NOTES ON

MICRO-ORGANISMS PATHOGENIC TO MAN

BY

SURGEON-CAPTAIN B. H. S. LEUMANN, I.M.S.

M.B. (London), D.P.H. (Cambridge), etc. etc.

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PREFACE.

THE object of the following "Notes" is to afford a clear and concise description of the life and growth of the chief pathogenic micro-organisms to those desirous of trying to understand the processes by which these micro-organisms produce disease. They will, I trust, supply a want to students and practitioners who have no opportunity of working at the subject themselves or time to read a larger book. They are based on lectures delivered at Netley during the Winter Session of 1893-94 by Professor Wright, who has very kindly allowed me to make use of the notes I then took, but who is in no way responsible for any errors I may have since introduced. On the other hand, I have considerably supplemented my notes of his lectures by extracts from various medical and bacteriological journals up to the present time and, by frequent reference to such well-known text-books as those of Flügge, Frænkel, Crookshank, Sims Woodhead, and others, have developed them into their present form. Nevertheless, my chief aim has been to attain brevity throughout—not with the idea of avoiding discussion, but in order to help the reader by not wearying him with too much matter; and if, in consequence, I may appear to be somewhat dogmatic in certain passages, it is not because I hold myself in any way an authority to speak definitely on the subject, but is simply an endeavour on my part to lay before the student what seems to me to be the most generally accepted and intelligible view of the case.

I have to thank M. Haffkine for his kindness in having revised the chapter on Cholera.

The account of Bubonic Plague contains a good deal of original work, not yet completed, on this disease. In the earlier part of the book considerable stress is laid on the "Phagocytic Theory" of Metchnikoff, while arguments against it are stated in the course of the work and discussed in Chapter XI. This has been purposely done as there is much in that ingenious and attractive theory which will probably help the student to grasp various facts, although the explanation of them so given may not be absolutely correct. In Chapter XI, also, I have made a few remarks on the treatment by, and practicability of prophylactic and curative inoculation fluids, but as such methods and adaptations are hardly out of their infancy, so to speak, I do not offer these comments as wholly final and conclusive.

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St. George's Hospital,
Bombay,
April 30th, 1897.

CONTENTS.

NOTES ON MICRO-ORGANISMS PATHOGENIC TO MAN

CHAPTER I.

MICRO-ORGANISMS exist almost universally—in the air we breathe, the water we drink, the food we eat ; while they are able to flourish in the upper layers of the soil. The mouth, nose, and digestive tract of man contains hosts of them: they live on the surface of our bodies, under the nails of our fingers and toes, in the external ear, within the conjunctivæ, under the prepuce, and within the vagina. In fact, wherever animals or vegetables live and die, there are micro-organisms present. The atmosphere on the heights of glaciers and glacier-ice itself have been shewn to be free of micro-organisms, but in all other places where man has searched for them there has he found them.

Those which concern us are the so-called "pathogenic micro-organisms," capable of producing disease, and although they as well as non-pathogenic forms may exist without disease occurring in such situations as the nose, mouth, etc., yet are not present in the juices or tissues of the normal body, but are only found therein when disease supervenes.

As their name implies, micro-organisms are tiny microscopical bodies, which, being free from Chlorophyll, require their Carbon in an organised form for food, and live either in the presence of water or by moist subtraction. They grow very rapidly, but if the medium be limited, they soon become absorbed or killed by the products of their own growth. According as they are capable of existing within their host, or of leading an independent existence without, so are they named Parasites or Saprophytes.

Micro-organisms may be classified as follows :—

I.—Animal micro-organisms—Protozoa.

II.—Vegetable micro-organisms—(*a.*) Moulds.
(*b.*) Yeasts.
(*c.*) Bacteria.

Characteristics of Protozoa. These are the smallest and most simple forms of animal life, having neither body-cavity, nervous system, sense-organs, nor any other differentiation of tissue. They possess the simplest (amœboid) power of movement, multiply by fission, and assimilate and excrete by processes comparable to endosmosis and exosmosis. Those which concern us are the Malarial Parasite, the Amœba Coli and the Cancer-bodies.

Characteristics of Moulds. (*Filamentous Fungi.*) These consist of a root-part or mycelium, and of hyphæ or threads, from which spore-bearing stems may grow. They require the least amount of water for their existence of all vegetable micro-organisms : proliferate relatively slowly by apical growth or by spores borne on a stalk: prefer acid media : are parasitic on plants and on lower animals, *e.g.*, insects, but rarely parasitic on higher animals, *e.g.*, man—except in such cases as Actinomycosis, Madura-foot, Favus, Thrush, Ringworm.

Characteristics of Yeasts. (*Sprouting Fungi.*) These consist of ovoid cells containing granules and sometimes vacuoles. They require liquid media : prefer an acid reaction : and proliferate by budding, the buds on being formed by the cells increasing in size and finally separating from their parents to reproduce further. Yeasts are essentially saprophytic, but the itching of the genitals that occurs in Diabetes is probably due to a parasitic yeast; while some consider the micro-organism of Thrush to be a yeast and not a mould.

Characteristics of Bacteria. *A. Classification.*—These minute forms of vegetable growth are classified according to their shape into straight rods called Bacilli, curved rods or Vibrios, and spheres or Cocci. Forms of aggregation due to growth are further known as under :—

Bacillus.	Leptothrix	=thread-form—cp. Anthrax.
	Separate, single	—cp. B. Subbilis.
Vibrio.	Spirillum	=Spiral form—cp. Relapsing Fever.
	Comma, separate.	
Coccus.	Diplococcus	= two together.
	Streptococcus	= chains.
	Staphylococcus	= Mulberry-like clusters.
	Tetrads	= 4 together (2 planes of division.)
	Sarcina	= 8 together (3 planes of division.)

B. Structure of Bacteria.—They consist of a delicate protoplasm which stains deeply with aniline dyes. It is supposed that nuclear substance is scattered throughout this otherwise homogeneous protoplasmic material since staining occurs more deeply in some places than in others, and indeed the presence of this substance

would account for the rapid division of the cell. Outside the protoplasm is a cell-membrane, of a substance allied to cellulose but not actually cellulose itself, and around this membrane is a gelatinous material enveloping individuals and serving to unite the bacteria with one another.

C. Power of Movement.—Many bacteria are motile, others non-motile. The movement is produced by flagella or cilia, placed respectively at the ends or sides of the bacterium. Some have numerous flagella situated more or less irregularly: while micrococci and certain bacilli—*e.g.*, B. Anthracis—have not been found to possess either flagella or cilia.

D. Method of Reproduction.—(*I*). *Fission or Division.* This method, under favourable conditions, may occur very rapidly, even every half-hour or so. Division in rod-shaped bacilli and some cocci takes place at right angles to the longitudinal axis of the micro-organism: but in some forms a more or less perfect division parallel to the longitudinal axis occurs, which gives rise to a branched appearance: in others again (non-pathogenic bacteria) the division may be in both directions. (*II*). *Spore-formation.*—(*a*) Under certain conditions the protoplasm of each bacterium becomes granular (*cp.* B. Anthracis), and later on a small bright spot appears within. This is the spore, and the method by which it is produced is termed *Endospore formation.* (*b*) Sometimes one individual amongst a group becomes modified for spore-formation, surviving when its fellows die, and producing a spore which will later on be able to reproduce the micro-organism by development under favourable conditions. This method is known as *Arthrospore formation. The spore* consists of an inner very refractile centre and a dark lining membrane, outside which is a gelatinous film.

E. Conditions of Growth.—(*I*). *A liquid medium* is necessary, for drying kills many bacteria, though some, possessing very thick capsules, appear to resist it. (*II*). *A slightly alkaline* or at least a *neutral* reaction of the *medium.* Even 2 per cent. Hydrochloric Acid is fatal to the majority of bacteria (Sarcina Ventriculi can, however, live in it), and though some non-pathogenic forms can live and multiply on Acid Phosphate of Sodium, stronger acid kills off all varieties. The "micrococcus ureæ," on the other hand, is capable of living in extremely alkaline urine. (*III*). *Electricity*, in a powerful current, passed through a culture of bacteria is said to kill them. (*IV*). Excessive movement retards their growth—either in a culture-tube, or in a swiftly-flowing river. (*V*). *A requisite temperature* is needed. Pathogenic bacteria grow best at the temperature of the human body—*i.e.*, at 37°C. They are able to resist cold better than heat, and though growth usually ceases at 5°C., some forms can survive intense cold. If, however, the temperature be raised to 45°C., the

growth of most bacteria will be arrested, and the micro-organisms themselves killed at 60°C. to 65°C. Spores are very resistant to dry heat, but can be destroyed by boiling (see below). (*VI*). *Food requirements and conditions for obtaining them*—(*a*) *Carbon* is needed in an organised state and is best obtained in the form of diffusible albumins. Bacteria differ from chlorophyll-bearing plants in not obtaining their carbon from the CO_2 of the atmosphere. They differ from animals in building up their protoplasm from comparatively small molecular groups. On the other hand, they agree with animals in possessing the power of breaking down organic matter into smaller molecular groups, though they flourish best when fed on food that does not require to be broken down to be absorbed. (*b*) A certain amount of *water*, as has already been stated. (*c*) Some bacteria require *oxygen*, and are hence called *aerobic*; others which do not being known as *anaerobic* bacteria, *cp.* the Bacilli of Tetanus and Malignant Œdema. (*d*) Those which live on or in higher bodies are termed "*Parasites*." Those which are able to lead a separate existence, "*Saprophytes*."

Some parasites, however, have never yet been cultivated outside their hosts. Such are called *Obligate Parasites* (*cp.* Leprosy and Relapsing Fever). Others again, though usually living outside, can, under certain favourable conditions, live in an animal host, and are known as *Facultative Parasites*. Further, *Obligate Aerobes* are those bacteria which can only live in the presence of oxygen; while *Facultative Aerobes* have or can develop the power of obtaining their oxygen from the medium in which they are cultivated (*cp.* the Bacilli of Enteric Fever and of Anthrax; Diplococcus Pneumoniæ; and Staphylococcus Pyogenes). In the same way we speak of *Obligate Saprophytes* or Saprophytes which under no conditions are able to exist as parasites (but these do not concern us in this book at all), and *Facultative Saprophytes*, which can. In connection with this, it may be noted that the Bacilli of Anthrax, Tetanus, and Malignant Œdema can, under certain conditions, become either facultative parasites or facultative saprophytes. (*VII*). *Sunlight* arrests the growth of bacteria and the conversion of spores into bacilli, etc., and may even kill them. The *electric arc-light* will act in the same way, but to a less degree.

F. Staining Properties.—Basic aniline dyes are usually readily taken up. Resistance to staining and drying go together, and depend on the nature or thickness of the capsule.

G. Certain diagnostic culture-properties.—Amongst these we may mention colouration products; the power of liquefying a gelatine medium, or not; the production of gases and odours; the production of phosphorescence; the action of precipitating casein in milk and dissolving it afterwards, or not; fermentative and putrefactive properties, etc.

H. Production of Toxins.—These are poisons, proteid (albumose) or crystalloid in nature, both which are formed in addition to the ordinary products of decomposition—*e.g.*, Ptomaines, Leucin, Tyrosin, Ammonia, Nitrates, Nitrites, down to the stages of CO_2, N and H.

I. Polymorphism.—Under varying conditions, the physiological characters of Bacteria alter as well as their forms, although the more perfect our methods of investigation become, the less differences shall we find, and the more easily shall we be able to establish the identity of the different forms.

Distribution of diseases produced by micro-organisms is seldom brought about by air-currents because the atmosphere is usually too dry. Thus, cholera is not blown about (so to speak) and the direction of the wind, *per se*, has probably little or no direct influence on the spread of the disease. But bacteria can be conveyed in moisture and on particles of dust, and hence air-currents may act indirectly. Being ubiquitous, or practically so, micro-organisms exist and thrive in the superficial layers of the soil, though they are not found at a depth greater than five or six feet below the surface. As has been said, they are found on the surfaces of healthy bodies and in the cavities and exposed mucous tracts, etc., of the body; but they are not present—in healthy individuals—in the mucous membranes of the bladder, uterus, Fallopian tubes, or urethra (except at the meatus). Hence diseases produced by these micro-organisms are distributed in the main by contagion either directly as from one body to another, or indirectly through the medium of food, drink, etc., and less often by air-currents.

Invasion of the human body by micro-organisms is effected by wounds and abrasions of the skin and superficial mucous membranes, and through the various external orifices leading to cavities and channels, digestive, respiratory, etc., in the body, and also through the placenta. In the nasal cavities, larynx, and other places, we find special arrangements of Nature—*viz.* cilia—on the surface of the cells, by which she contrives as far as possible to exclude these micro-organisms and to brush them away even if they have managed to pass the barrier of moist mucous membrane at the oral and nasal orifices. Transference within the body occurs either by the blood or lymph channels or by both.

The predisposition of the organism to the micro-organism is a marked factor in which racial, individual, and tissue peculiarities must be considered as well as other causes that predispose, and the general condition of the organism, facts which we will enter into further on when discussing the subject of immunity.

Diseases due to micro-organisms generally may be classified as—(*I*). *Infectious diseases*, the micro-organisms penetrating the body from without and not necessarily by contact; (*II*). *Contagious diseases*, in which the micro-organism passes directly

from the body of an infected organism into that of a non-infected organism in which it proceeds to produce the disease afresh. (*III*). *Miasmatic diseases* ; the micro-organism is here discharged from an infected body on to the soil or water, directly or indirectly, where it can live and whence it can be introduced into another body and reproduce the disease.

REMARKS ON STERILISATION.

Heat is most efficient for this purpose ; either dry heat, when a high temperature is required ; or moist heat, when a lower temperature will suffice ; but it must be remembered that to "sterilise" properly, the spores as well as the full-grown micro-organisms must be killed. This can be done by a dry temperature of 170°C. applied for 10 minutes, or 150°C. for 30 minutes ; or by a moist temperature (air saturated with aqueous vapour) of 115°C. for half-an-hour ; or by a saturated moist heat of 100°C. applied for 3 hours. Pressure increases the effect if used in addition to moisture and heat ; thus, superheated air supersaturated with aqueous vapour under increased pressure is used for the disinfection of infected clothes, rags, etc. In bacteriological work such high temperatures often cannot be used as they would destroy the nutrient substance on which the micro-organisms are grown, *e.g.*, gelatine. In such cases the culture-medium is exposed for a quarter of an hour to steam whereby all the adult micro-organisms are destroyed. It is put aside for 24 hours, after which time the spores that were left behind have developed into bacteria proper, and then similarly treated, and the process repeated a third time. In this way all spores have developed into full-grown micro-organisms and have been destroyed as such. This is known as *Intermittent Sterilisation.* Or, dry heat may be used in the case of gelatine, the substance being first heated to 75°C., then cooled and left ; reheated twice to 70°C. with an interval interposed, after which all the spores will have been destroyed. Filtration through a Pasteur-Chamberland filter, with or without heat, may also be used for the sterilisation of culture-media.

CHAPTER II.

To pass over in review the various stages by which the causal connection of micro-organisms with disease has been established and proved, would be beyond the scope of a small work of this kind, and may indeed at the present day be regarded as a chapter from ancient history, since such proof has been established beyond all possibility of doubt, and is now everywhere accepted. In order to be certain, however, that a given micro-organism is the specific cause of the disease attributed to it, it is recognised as essential that the three laws or canons enunciated by Koch shall be obeyed and satisfied first.

Koch's Canons.

I.—The particular micro-organism to which the disease is attributed must be found in every case of the disease and in no other case.

II.—The micro-organism in question must be cultivated for a number of generations apart from the animal organism.

III.—The micro-organisms which have been thus grown must be able to reproduce the disease identically when introduced into other organisms must be found in them and be capable of being grown apart (*i.e.*, repeating Canon No. II.) again.

The difficulties met with in establishing this chain of evidence are, as can be imagined, neither few nor similar, for instance :—

As to Canon I.—Difficulties in isolating the micro-organism when in relation to the disease are found—(*a*) in identifying the micro-organism in the unstained condition, either in sections or in cover-glass preparations ; (*b*) in staining the micro-organism when identified—thus unstained spores and bacteria will be invisible in a stained section ; or if stained the same colour as the background or nuclei of the cells, they will still not be visible, and hence must be stained a different colour to be distinguished ; and (*c*) in identifying a particular micro-organism amongst others adventitious, examples of which are seen in Typhus Fever, Vaccinia, Measles, Tubercle, Diphtheria, Cholera, Enteric Fever, etc.

As to Canon II.—Difficulties in growing the micro-organism apart from the organism—(*a*) in finding a medium in which the micro-organism will grow. Different media must be successively

tried until a suitable one is found ; (*b*) in finding a medium in which the micro-organism in question will not be out-distanced by competing adventitious ones :—instances of this occur in Tubercle, Influenza, Enteric Fever, Diphtheria, etc. ; and (*c*) difficulties—in the case of anaerobic micro-organisms—associated with atmospheric oxygen (*cp.* Tetanus).

As to Canon III.—Difficulties in finding a suitable animal in which to reproduce the disease. This involves the general question of the zoological evidence of disease, for instance—(*a*) Cholera, Relapsing Fever, and Gonorrhœa appear to be purely human diseases ; (*b*) only man, tortoises, and birds are known to suffer from Malarial Fevers. The difficulty may be met and turned either—(*i*). by diminishing the resistance of the animal—*cp.* Koch's method of giving Cholera to guineapigs by first administering opium ; (*ii*). by exalting the virulence of the micro-organism, as by making successive cultures until a certain maximum is reached ; or (*iii*). by employing mankind for experimental purposes—a method which is full of objections and difficulties.

Classification of Bacterial Diseases. While diseases due to micro-organisms generally may be conveniently classified into Infectious, Contagious, and Miasmatic—as has been discussed above—those caused by Bacteria may, from a pathological point of view, with still greater usefulness, be grouped according to their effects in animals most susceptible to them. Thus we recognise three varieties :—

I.—General Septicæmic Processes.—In such cases the micro-organisms may not be discovered at the seat of inoculation, but are found *post-mortem* swarming throughout the body.

II.—Local Destructive Processes.—Each of these marks a course favourable to the animal affected, since it shows a local attack by micro-organisms, and then their subsequent destruction: in fact the resistance of an animal to a micro-organism may be said to vary with its power of local resistance to an attack. A local destructive process may, however, pass on into a general septicæmia—either through the blood (as in Anthrax attacking man), or more readily still by the lymph-channels (as is seen in Streptococcus Pyogenes—first an abscess and then a general septicæmia occurring).

III.—Intoxication Processes.—The products of the micro-organisms here are more poisonous than in either of the former two groups, and being alone absorbed into the body—the micro-organisms themselves not entering—there produce their effect.

Although convenient for classification purposes, it is in [illegible]ny instances not possible to draw any hard and fast line be[illegible]ese forms of disease, nor when one has practical acquain[illegible]th them from the standpoint of treatment, as will be seen

when such diseases as Anthrax, Enteric Fever, Cholera, and Diphtheria come to be considered; for the virulence of the micro-organism and its attack must be taken into account as well as the susceptibility of the organism. With regard to this latter item, witness the fact that from a trivial "poisoned" wound of the finger one patient may develop a general septicæmia without any local effect to speak of, while another will get a whitlow—but nothing further—from a quite similar injury.

Thus, local reaction on the part of the organism is its safeguard against bacterial attack. If a healthy rabbit be inoculated hypodermically with ordinary pus containing Streptococcus Pyogenes, only a local abscess results: but if the animal be first kept in cold water for some time, so that its vitality is lowered, or if the vasomotor nerves leading to the part be cut instead—then no local reaction in the form of an abscess is seen, but a general septicæmia occurs. And in connection with these remarks, it may be noted that if Anthrax be inoculated into a rabbit, a general septicæmia results; but in man a local destructive process—the malignant pustule—first manifests itself. So by considering the zoological incidence of the disease, one learns how it is that a particular bacterial infection of given strength in one case takes the form of a general septicæmia, in another of a local destructive process, and may, in a third, give rise to an intoxication.

Nature of the Toxins produced by Bacteria.

The toxic products of bacterial cultures have often been found to be capable of separation into two component parts—crystallisable and non-crystallisable. These latter are of the nature of proteids—tox-albumoses, tox-albumens, or simpler. They are produced either by the result of the action of the bacteria on the surrounding media; or they may be bound up in the bodies of the bacteria and only set free in old cultures or by treatment with alkalies. In some cases no crystallisable alkaloid can be made out—*e.g.*, in Diphtheria toxin; in others it is presumed to exist as a sort of enzyme or organised ferment bound up in the composition of nucleo-albumins, globulins, albumoses, or allied bodies. Whatever be its nature, toxic material not only is capable of exerting an attractive power ("positive chemotaxis") on the leucocytes of the blood, but also of causing them to degenerate and die after arrival—pus frequently being formed.

CHAPTER III.

THE QUESTION OF IMMUNITY.

UNDER this heading we consider how an animal gets, suffers from, parts with, and is cured from, a particular disease. Now, each animal may be said to have his own particular diseases, and to be insusceptible to other diseases ; while again, two similar animals may react differently when exposed to the influence of a disease to which their class is liable—for instance, a white mouse is readily killed by a dose of Anthrax which a house-mouse is easily able to successfully resist. In an outbreak, say, of small-pox or scarlatina, one man suffers, another does not. What is the explanation of this well-known fact? Even the vulgar mind is fully aware that one attack of an exanthematous disease in the vast majority of cases protects against another (more or less completely), so that the man who escapes may have been previously protected by a former attack or in some other way, or he may be looked upon, perhaps, as having some hereditary or racial protection against the disease, which in the other man obtains a foothold by reason of his hereditary or racial susceptibility. On the other hand it is generally considered that an attack of Diphtheria or Asiatic Cholera confers temporary immunity, but that after this wears off the susceptibility of the organism to those diseases becomes greater than before ; though in the case of Cholera, at any rate, this account must be received with caution, for no statistics have been put forward to show that one attack does not guard against another, and, in fact, recent investigators are inclined to think that on further enquiry this may be found to be actually the case, but for the present nothing is definitely known about it. Immunity may be altered or destroyed by artificial means. Thus, if Phloridzin be given to white mice glycosuria is produced, and they become immediately susceptible to Glanders, to which they were naturally immune before. Again, Anthrax bacilli subcutaneously injected in the frog produce no local inflammation as active phagocytosis occurs at the site of inoculation ; nor will there be any local result from a similar injection of Tetanus bacilli in the human subject, although in this case the poison which the bacilli will form will be fatal. The whole problem

needs experiment for its solution, and further knowledge is necessary for us to be in a position to render animals artificially immune against diseases with definite certainty.

Immunity may be defined as the power of the organism to either prevent the entrance of pathogenic micro-organisms, or if they have entered to prevent their further growth, or the development of their poisonous properties. *Susceptibility* may be regarded as the passive state of the organism favouring exactly the opposite conditions to Immunity.

Immunity may depend upon animals naturally being or artificially becoming or being made either—(*a*) *Poison-proof*, or (*b*) *Bacteria-proof*. The method of artificial immunisation depends upon which of these ends is required to be accomplished in a particular case. For instance, in an intoxication process, like tetanus, the animal is practically proof against the micro-organism. We must, therefore, aim at making it, either directly or indirectly, poison-proof. Similarly, in other cases the converse holds good, *i.e.*, we must render an animal bacteria-proof if it shows signs of being poison-proof.

The condition of being poison-proof may depend upon—(*I*). *A natural insusceptibility*, as is seen in the case of fowls against tetanus-poison. (*II*). *Tolerance obtained by Mithridatism*, such as the tolerance to tetanus-poison obtained in rabbits by increasing the successive doses of tetanus-poison administered to them. With this, compare the acquired tolerance obtained, after a time, of certain poisons, such as Tobacco, Opium, Arsenic, by man. (*III*). *An antitoxic property of the humours of the body*, such as the blood-serum and the milk. Ehrlich found that rabbits could obtain a tolerance of certain vegetable poisons—aborin, ricinin, roborin, etc. ; and further that the serum of the animals which had been so treated had become antagonistic to the action of tetanus-poison. In animals this developed antitoxic power of the humours may become hereditary : and also be imparted by the milk from the mother. In all cases where antitoxin is produced, it must be remembered that it is an entirely new substance (proteid in nature), and does not occur in the blood of normal animals. The antitoxin is a protective substance formed by the energy of the tissues, not depending on the presence of the bacteria, but of the poison produced by them ; and it is not harmful or fatal to the bacteria themselves, but counteracts the effect of the poison they produce—in other words, it practically renders pathogenic bacteria non-pathogenic by hindering and counteracting the formation of their poison. An antitoxin is only of use against its own specific poison ; thus, diphtheria antitoxin is of no avail against tetanus-poison. Such is a rough sketch of the *Theory of Antitoxins*. (*IV*). *Upon an attenuating power of the humours*, causing an inhibition of the

production of the poison—as when Anthrax is grown on the serum of sheep. When such diseases as Small-pox, Scarlet Fever, etc., invade the body, it is supposed by many that either some substance is originated in the humours which is prejudicial to the further growth and development of the micro-organism (*Retention Theory*, and there appears to be a good deal of truth in this); or that the micro-organism uses up some substance and so cannot develop further (*Exhaustion Theory*). This latter theory, however, would mean that there is a certain material in the body ready for each disease; while the former theory asserts that a certain material is left behind in every case. Either view necessitates too complex a chemical state of the blood and other humours to be accepted *in toto*.

The condition of being bacteria-proof may depend upon—(*I*). *A bactericidal property of the humours—e.g.*, the plasma. This is tested by repeated time-growths, made from one original culture of bacilli in a tube of serum, on successive plate cultivations, not from one plate to another, but from the original medium in the tube to the new medium in each new plate. By this means the number of bacteria are found to decrease in successive plates. The fallacy of the method employed is that there is a change of medium, and serum has been employed instead of plasma. Again, in many cases, the serum of immune animals does not possess bactericidal power. When it was first noticed that Anthrax bacilli were at once killed on being introduced into a few drops of rabbit's blood, the above theory—*Humoral Theory*—gained much credence, and has lately been revived; but it was shewn that in shed blood, many white corpuscles get broken up and destroyed, and their nuclear matter being set free is fatal to the bacilli, and further, it has been proved that spores of Bacillus Subtilis can remain uninjured in the spleen—an organ which is full of blood—for as long as three months. (*II*). *A phagocytic action of the cells.* Metchnikoff first showed that "phagocytosis" or the swallowing and incorporating of bacteria or other small particles, living or not, by certain cells of the body, occurs. Such cells are termed "*phagocytes*," and may be *fixed*—as endothelium, connective-tissue cells, etc., or *free*—as the white corpuscles of the blood, but all possess amœboid movement. Those which concern us most are the white blood-cells. On examination, four varieties of white corpuscles are to be met with in the blood—(*a*) large granular cells, which have a horse-shoe shaped or irregular nucleus and are eosinophilous. Latest accounts (Kanthack and Hardy) state that these are of two kinds—in one the granules are coarse and the nucleus is shaped like a horse-shoe; in the other the granules are fine and the nucleus is an irregularly branching mass; (*b*) finely granular basophile cells, whose nuclei are split up into 2 or 3 lobes; (*c*) hyaline cells, large and free from granules, containing a spherical nucleus; and (*d*) lymphocytes,

the smallest of all, whose nucleus occupies nearly the whole of the cell and is surrounded by a thin layer of protoplasm only. In some of the above forms, *e.g.*, (*a*) and (*b*), the nucleus, instead of being divided into lobes, appears to be in distinct pieces, which earns these cells the name of the polynuclear leucocytes of the blood. If anthrax bacilli be injected into the frog, the eosinophilous cells are seen to come and attack them first, perhaps swallowing them wholly or in part. The hyaline cells aid to the best of their ability by incorporating the remains of the bacilli which the eosinophilous cells have destroyed. The power in these latter cells to kill the bacilli seems to be in their eosinophilous granules, for these can be seen to dissolve, and so probably exert their action on the bacilli. Lastly, the basophile cells, whose granules are supposed to counteract the poison of the bacilli, come up—and behind them the lymphocytes—and enclose the area of combat. All this may be very prettily shewn by a simple experiment. If some carmine (previously dissolved in an alkali and then precipitated by an acid) be injected into the marginal vein of a rabbit's ear, and after the lapse of 15 to 30 minutes, the blood of the animal be examined, then only mononuclear leucocytes are found in the blood stream, but no polynuclear leucocytes. On searching in the spleen pulp, however, the latter are found in crowds, filled with carmine granules which they have picked up in the blood stream and carried off bodily to that organ.

Three chief objections against this Phagocytic Theory have been raised:—(*I*). that a ridiculous amount of intelligence has been ascribed to leucocytes ; (*II*). that the bacteria are not really living, but are dead when taken up by the leucocytes ; and (*III*). that the bacteria get the better of the cells.

Examination of these objections and replies.—(*I*). There are numerous vegetable organisms known which are attracted by certain chemical substances, so there is no reason why certain structures, *e.g.*, bacteria, should not attract leucocytes, more especially as the bacterial products attract as well as the bacteria themselves. Further, leucocytes shew this amount of intelligence, that they "evince a distinct selective tendency between various kinds of organisms" (Ruffer), and if Bacillus Prodigiosus be introduced simultaneously with the Tetanus Bacillus, they will leave the latter for the former. (*II*). Amœbæ feed on small particles, so even if dead the bacteria may be regarded as food particles for the leucocytes, which thus shew a certain amount of intelligence in taking them up at all ; but that they can and do take up living bacilli Metchnikoff has been able to prove by obtaining a growth of anthrax from bacilli contained within leucocytes. (*III*). Bacteria may certainly get the better of some cells, but they do not vanquish all, being in turn destroyed by other leucocytes which come up to the rescue, and so the battle is waged till one or other side wins. A tubercle

bacillus, being more powerful than a leucocyte, may very likely kill it, although the leucocyte can swallow the tubercle bacillus almost as easily as it can swallow carmine particles. This shows that phagocytosis occurs, but does not explain its value; so let us compare the amœba, which lives on bacteria, with the leucocyte, as an intellectual equivalent. If the bacteria are too powerful for the amœba, it is killed and eaten by them and not they by the amœba. This is the very beginning of Parasitism, and is a question of whose digestive juices are the more powerful—if the amœba's then the bacteria die, and *vice versa*. Now, it is not merely a question of size, for if we trace the development of Protozoa into Metazoa, we find that specialisation of functions—not organisation of new functions—has occurred; and consequently, we can consider the relations of Metazoa and Protozoa to bacteria from the same standpoint. Thus, Metchnikoff found that the water-flea (Daphnia Pulex) becomes infected by a parasite called Monospora, whose white fungus-spores are clearly visible in its transparent body, and can be seen to be attacked by leucocytes which take the majority into their substance and destroy them, but other spores, escaping their attack, germinate and grow in the water-flea's body. Since, then, animal cells can take up bacteria, and the amœba can take up and digest even leptothrix forms many times as long as itself, there is every reason to accept Metchnikoff's view of Phagocytosis. The leucocytes will less readily attack a powerful than a weak micro-organism (for the reasons given below), and their action will always be more aggressive to bacteria in an immunised body than in a susceptible one.

Chemotaxis.

What brings the leucocytes at all to the point of bacterial attack? If a bit of aseptic cotton wool be aseptically introduced under the skin of an animal no result, so far as the leucocytes are concerned, is observed; they are neither attracted to the cotton wool nor repelled by it. But if a dilute solution of turpentine had been placed on the cotton-wool before its introduction, leucocytes would then be found around it and even entangled in its meshes. This power of drawing the cells together is called *Chemotaxis*, and when it attracts the cells that are able to repulse, or wish to devour, it is called *Positive Chemotaxis*. If the cotton-wool has been saturated with pure instead of diluted turpentine, no leucocytes will be found within its meshes, though some few may be lying dead around, but the majority will keep at a very respectful distance, being repelled by *Negative Chemotaxis*. In the case of a local inflammatory process the resistance to the bacterial invasion requires to be rendered more complete. In fact the clinical symptoms of local inflammation are all expressions of inefficient local resistance.

The theories of Immunity may be tested by making a naturally susceptible animal immune, and then testing what new properties have been acquired by its humours and cells. If a culture

of Vibrio Metchnikoff (a kind of cholera that affects some animals) be boiled so that the bacteria are killed, and a dose of the sterilised products (Chemical Vaccination) be inoculated into a guineapig, it is found that on inoculating it with the Vibrio Metchnikoff itself it does not die, while another guineapig which has not been previously treated in this way does. If now a third guineapig be inoculated, after having been first vaccinated with serum from an immunised animal, it does not die either. On examining the leucocytes of the three guineapigs, those of the second animal are found to contain a few bacilli, while the leucocytes of the first and third contain a large number; but on the other hand, colonies made from the blood of these guineapigs shew marked growth of the bacilli in the second, but none or only a very few in the first and third. In other words, Mithridatism of the leucocytes, not of the organism, has occurred. Bacteria grown on the serum of a vaccinated guineapig will not kill another guineapig, as they have become attenuated. The serum owes this property to the former treatment of the animal, and possesses bactericidal power, which is not found in the humours of the living body and hence was probably introduced into the serum by the disintegration of leucocytes when the blood was shed.

Immunity may further be considered as to whether it be required to be artificially produced against Septicæmias, Local Destructive Processes, or Intoxications. Thus :—

I. *Immunity against Septicæmias*, produced by methods which directly or indirectly render the animal bacteria-proof.—(*a*) Infection of an animal by the bacillus which causes the disease.—*Bacterial Vaccination.* This is based on clinical experience of immunity after previous attacks of diseases, and is always brought about by attenuated virus—*cp.* Inoculation with mild Small-pox; Vaccination against Small-pox as it is now performed; Pasteur's inoculation against Anthrax. (*b*) *Chemical Vaccination* by sterilised cultures of the micro-organisms. (*c*) *Preventitive Serum.* (*d*) *Vaccination by mixed infections* (antagonistic micro-organism). II. *Immunity against Local Destructive Processes* is hard to produce, and is theoretically slight. This will be considered later on. III. *Immunity against intoxications* is produced by making the animal either directly or indirectly poison-proof. (*a*) *Mithridatism*—small, non-fatal doses at first, succeeded by larger ones as tolerance is obtained. (*b*) *Method of Antitoxins*—(*i*). Filtrate freed of germs; (*ii*). sterilised cultures containing the germs; (*iii*). serum obtained from animals rendered immune in any way.

It must be remembered that the above is a purely theoretical consideration, and that methods differ in practice. For instance, in Anti-Cholera Vaccination (Haffkine) the pure comma-bacillus is injected subcutaneously, and no absolutely certain method by antitoxin for the treatment of the disease in man is yet known. This

is because the toxin of Cholera is so intimately connected with the comma-bacillus as to be, for all practical purposes, almost absolutely inseparable by filtration, etc. Hence the pure growth of the micro-organism has to be used, and this method by rendering the animal bacteria-proof indirectly (in theory, but in practice also directly—since the toxin is so inseparable from the comma-bacillus) renders it poison-proof.

No matter how immunity has been produced, the following points are worthy of note regarding it—(*i*) Sterilised cultures and filtrates freed of bacteria and rendered non-poisonous, have a marked effect in producing immunity; (*ii*) serum immunisation often appears far more quickly, but is never so lasting as that produced by the bacterial method; (*iii*) if bacteria are used, a mild attack is the end to be kept in view—after which immunity will be established; (*iv*) after inoculation the animal appears to be for a short time more liable to the disease from which it later obtains immunity—at least in some cases; (*v*) the serum of organisms naturally immune has not yet been satisfactorily proved to be able to confer immunity on organisms naturally susceptible; and (*vi*) the nature of the immunising substance is proteid.

Natural Immunity in animals may be destroyed—(*i*) *By causing a variation of the normal temperature.* In this way Pasteur caused chickens to take anthrax, to which they are naturally immune, by giving them a cold bath first, and inoculating them with anthrax afterwards; (*ii*) *By altering the composition of the blood.* Leo found that by injecting phloridzin into white rats or administering it to them as food, the animals lost their immunity against anthrax; while Hankin obtained the same end by feeding them exclusively on bread; (*iii*) *By removal of the spleen*—an operation, however, which of itself is frequently fatal; (*iv*) *By a mixed infection.* If Bacillus Prodigiosus be mixed with the Bacillus of Malignant Œdema and the two subcutaneously injected together into calves, they are said to suffer from the latter disease, though naturally immune against it.

CHAPTER IV.

Septicæmic Processes.

In true primary Septicæmias no reaction at the site of inoculation takes place, and there is always a period in the disease when phagocytosis fails to occur properly in the blood-stream and when bacteria are found free in it.

ANTHRAX.

Distribution—(*i*) *Geographically*, Anthrax is met with all over the world, being most common in Siberia, rare in England, and comparatively rare in America; and (*ii*) *Zoologically*, there are a very large number of susceptible animals. Cattle and sheep suffer worst of all, but horses, guineapigs, rabbits, and mice are also prone: and especially the young of all susceptible animals. Dogs, cats, amphibians, and most birds are practically immune; while white rats are infected only with difficulty.

Discovery of the disease.—Davaine in 1863, by inoculating the blood of anthrax-infected sheep into other sheep, and reproducing the disease typically in the latter, satisfied Koch's first canon; and Koch himself many years afterwards managed to isolate the bacillus. Both he and Pasteur grew it in pure cultivation, and then by injecting it into suitable susceptible animals from whom the bacilli were afterwards obtained, succeeded in satisfying the remaining two canons.

Morphology.

A rod-shaped, rectangular, non-motile bacillus, without flagella, occurring when in the blood of the living infected animal as short single or jointed segments, 6μ—9μ or more long, and about 1μ broad, with cup-shaped ends—and never, under such conditions, containing spores. When cultivated in artificial media, long filaments and bundles are formed in which the individual bacilli can be distinguished, adhering end to end. Under certain conditions, and in old cultures, spores are formed within the bacilli as ovoid transparent bodies which do not cause any bulging or other alteration of their shape.

Growth. Almost any medium, if slightly alkaline, will serve for the cultivation of the Anthrax bacillus, but a trace of free acid prohibits its growth. On gelatine plate-cultures characteristic colonies are formed, appearing as whitish dots on the surface and causing liquefaction of the medium, which becomes cloudy at first, but clears afterwards. Under the microscope, these colonies appear ovoid, brownish in the centre, and white on the surface, where growth is most rapid, and in the form of fur-like tufts composed of threads of bacilli. In a gelatine tube-culture produced by puncture the bacilli spread out horizontally, like the branches of a tree, all along the track of the needle, into the gelatine, so that the growth resembles an inverted pine-tree. On Agar-Agar these characteristics are not nearly so marked, but in this medium spore-formation is most luxuriant. In broth a fluorescence is seen at the bottom of the tube. Thus, Anthrax shows both aerobic and anaerobic growth. The temperature required must be not less than 16°C. nor more than 45°C.; that of the blood, *viz.*, 37°C., being the best.

Staining Reactions.—Basic aniline dyes are readily taken up. For staining the bacilli in tissues Gram's method or Weigert's fibrin method (*see* Chapter X.) give excellent and beautiful results. The spores, in a coverglass preparation, can be shewn well in contrast by staining them with Carbol-fuchsin, decolorising the bacillus with a very weak acid, and then staining with Methylene Blue.

Conditions of Spore-formation. As the presence of Oxygen is necessary, Anthrax must be grown on a surface, such as Agar-Agar, for this purpose. The best temperature is 25°C. but spore-formation can also occur between 20°C. and 33°C. No spores are formed in broth, or in the blood of living animals, though they will rapidly develop in the discharge from dead bodies. The spore begins to appear as a small point which enlarges until it nearly fills the whole bacillus. It has a very thick capsule, and hence is resistant to drying and difficult to stain, Loeffler's Blue being of no use as it cannot penetrate sufficiently.

Anthrax Spores are used as a test for the efficiency of Antiseptics, since they will remain virulent for many years if kept dry. The method employed is to take threads on which the spores have been allowed to dry, and immerse them in the antiseptic solution to be tested for a certain time; after which a plate cultivation is attempted from these threads, and it can be seen whether the spores have been destroyed or not. A temperature of 100°C. applied continuously for 5 minutes, or 5 per cent. Carbolic Acid solution for the same time, kills the spores, which can be destroyed

at once by 1 in 10,000 Perchloride of Mercury solution, and distinctly modified by a temperature of 50°C. kept up for two hours, or by sunlight, or certain chemical agents.

An Asporogen variety of Anthrax can be artificially produced by the addition of 1 in 1,000 Carbolic Acid solution to the nutrient medium in which it is grown.

Channels of Infection. (*I*) *Cutaneous wounds* and scratches (*cp.* Woolsorters' Disease amongst Mankind), and probably also by the bite of insects. (*II*) *The intestinal canal.*—The bacilli are of course killed by the acid gastric juice, but the spores are able to resist it and pass on to the alkaline contents of the intestine where they develop into bacilli which grow into the villi, between the epithelial cells, and having entered the lymphatics produce a general infection. (*III*) *The Lungs.*—If anthrax bacilli be sprayed into the mouth of an animal, Pneumonia results by pulmonary infection. If, however, the desiccated spores be employed instead, no pneumonic process but a general septicæmia of typical anthrax ensues.

Propagation of the Disease. Amongst flocks and herds of animals the disease is propagated by inhaling or swallowing the spores from off the grass or other pasture on which they have been deposited by the urine and bloody fæces of infected animals before they died. The dead bodies of the infected animals are not so great a source of danger, though by no means innocent of the power of infection. Consequently, all stools and urine should be thoroughly disinfected, and any animal found suffering from anthrax at once removed from its fellows and killed, —the carcass being burned, or buried at least 6 feet below the surface of the soil. Infection by wounds is, of course, possible in animals, but is probably much rarer than in mankind.

Course and Classification of the Disease. (*I*) *At the site of inoculation.*—In man a local destructive process—the malignant pustule—occurs; but in some animals there is very little local reaction, and in others none at all. If the infection is alimentary or respiratory, pustules may or may not form, and systemic symptoms may or may not occur. (*II*) *Systemic Infection.*—Access to the blood is obtained, either—(*a*) through direct ulceration into the capillaries, or (*b*) by means of the lymphatic system. (*III*) *Septicæmic Stage.*—This may be primary, as in cattle, sheep, and rodents, and in fact most animals, or—as when following on the local manifestation (in man and a few animals)—secondary.

Phagocytosis in Anthrax. As soon as the disease reaches the blood, phagocytosis occurs, and the bacilli are removed to the internal organs; the polynuclear leucocytes disappearing from the blood stream and accumulating in

the spleen and liver. Three stages can be made out in an Anthrax-infected rabbit. The first, which takes about 7-8 minutes, marks the elimination of the bacilli from the blood and their inclusion in cells in the liver and spleen. The second stage is a stationary period, during which the phagocytic process just balances the multiplication of the bacilli. The third stage records the excess of reproduction of the bacilli over the phagocytic phenomena. Now, it is found that the phagocytes are relatively more in number in the liver than in the spleen, and appear to be more effectual; which means, conversely, that the multiplication of the bacilli is more marked in the spleen than in the liver.

Inoculation Experiments. If a guineapig or rabbit be aseptically inoculated with Anthrax, no reaction in the form of leucocytosis occurs locally, but the animal suffers from anorexia, debility, and fever; sometimes bleeding occurs from the nose and rectum; and considerable œdema ensues round the point of injection. A septicæmia resulting, death occurs in 24 to 60 hours; and *post mortem*, besides the local œdema already noted, the liver, spleen, and abdominal organs generally are found enlarged and congested, and the lungs and heart engorged with blood. Coagulation has occurred in the blood-vessels, where crowds of bacilli are seen on microscopical examination—especially in the capillaries; but they are also found in the lymphatics, and sometimes in the glomeruli of the kidney, as meshes of long threads. The blood coagulation may be due to the enormous production of polynuclear leucocytes that follows the inoculation.

If Anthrax be inoculated under the skin of a frog—which animal is immune to the disease—and later on the lymph in the dorsal lymph-sacs and vessels be examined, leucocytes are seen containing anthrax bacilli in their substance.

Immunity in Anthrax is attributable to the condition of being or becoming bacteria-proof. The method suggested by the immunising effect of a previous attack in the case of specific fevers is most risky of application here. Natural immunity against the disease can be destroyed, for Pasteur found that by giving fowls a cold bath immediately after inoculating them with anthrax, their immunity was lost. Again, if a frog be kept at 37°C. after inoculation it will "take" the disease—phagocytosis being apparently prevented, in the frog, at this temperature; and further, if an immune animal be inoculated with Bacillus Prodigiosus and Bacillus Anthracis together the latter micro-organism will exert its effect, presumably because the leucocytes shew preference for the former.

Hence there is necessity for attenuation in artificial immunisation, for Mithridatism being of practically no avail in Septicæmias, the only method is to render the animal bacteria-proof, either the humours or (more probably) the cells becoming bactericidal.

Methods of Attenuation.

I. *Pasteur's Vaccination.*—This process consists of two vaccines—(*a*) a weak preparation of anthrax bacilli, which have become attenuated by being grown at 42°C., of such a strength that a certain dose will kill a mouse but not a guineapig. In this case the bacilli are destroyed at the seat of inoculation, and there is practically no generalisation throughout the system. (*b*) A stronger preparation, obtained by growing anthrax at a lower temperature, such that a certain dose will kill a guineapig but not a rabbit. The process is one of slow chemical vaccination by bacterial products. In India it was found that, for horses, a weaker first vaccine was necessary, as that given above caused considerable œdema at the point of injection ; but Roux attributes this fact to the first vaccine having been probably made stronger than it ought to have been.

II. By the addition of ·2 per cent. Potassium Bichromate solution to the culture-medium. (Pasteur and Chamberland.)

III. Method of "*mixed infections.*"—Streptococcus Pyogenes being injected at the same time as the anthrax bacilli. This process is not properly understood.

IV. If 1 per cent. Carbolic Acid Solution be added to the blood of animals dead of Anthrax, the virulence of the bacillus is destroyed when taken from such a source and grown on a culture-medium.

V. *Serum-therapy.*—If into a susceptible animal the serum of the white rat, which possesses marked bactericidal power on anthrax bacilli, be injected, the animal is rendered immune ; but this occurs only if the white rat's serum is injected at the same time as and along with the anthrax bacilli, but not otherwise. Now, the white rat is not absolutely though nearly naturally immune, so the bactericidal property is not in its humours, but is produced during coagulation of its blood, and probably by the disintegration of its white corpuscles. This serum also has marked chemotaxic properties.

VI. Injection of Thymus extract (Wright)—but this process does not appear to have been completely worked out yet.

VII. By inoculation into the frog or other immune animal.

SPIRILLUM FEVER.

Discovery.—Obermeier in 1873 discovered the Spirillum which has since been found in all cases of so-called Relapsing Fever and under no other conditions. (Koch's First Canon fulfilled.)

Morphology.

This micro-organism is always found as a spirillum, 20–40μ long, and ·1μ broad, which takes basic stains with difficulty and will not stain by Gram's method. It possesses vigorous power of movement by means of flagella.

Conditions of Growth. These are unknown (*cp.* Leprosy) and all attempts at artificial cultivation have hitherto been unsuccessful. (Hence Koch's Canons Nos. II and III. have not been satisfied.)

Infection.—The mode of entrance of the spirillum into the body is uncertain as there is no local inflammation at the site of inoculation. It may, however, occur through *post-mortem* wounds, abrasions, etc.

Inoculation Experiments. The disease can be given to man and monkeys by inoculation of blood containing the spirilla.

Method of Preserving the Spirillum Alive.—When Relapsing Fever is at the height of one of its attacks, the blood containing spirilla can be extracted by leeches, in whose bodies they will continue to live ; and by this means the spirilla may be transported.

Facts relating to the Invasion Period and Crisis. When the fever first begins the spirilla are sparingly present in the blood, but more are seen as it increases in intensity, most being found when it has reached its height. They rapidly decrease in numbers as the febrile attack ends and are not found in the intervals between attacks. An increase of white corpuscles in the blood is to be noted synchronously with the increase in the number of spirilla. The liver and spleen become gradually larger and more painful. Then comes the crisis when all the spirilla and most of the white corpuscles disappear from the blood, but the liver and spleen remain enlarged and painful, thus shewing that phagocytosis has probably occurred. At this time (crisis) epistaxis is common and other hæmorrhages may occur—due no doubt to the corresponding diminution of the coagulability of the blood produced by the sudden withdrawal of the white blood-cells ; while œdema of the ankles, diarrhœa, and occasional eruptions also bear witness to its altered condition. The same communication may be traced—though somewhat less definitely—in rabbits, between the crisis of pneumonia and phagocytic action—and it may be that a crisis marks the termination (more or less complete) of a septicæmic process—*i.e.*, it marks some critical stage in the battle between the micro-organisms and the organism.

Histological Changes in the Spleen.—Miliary lymphomata occur, and the Malpighian bodies increase in size. Spirilla are found both free and contained in leucocytes in the spleen of a monkey about ten hours after their disappearance from the blood.

INFLUENZA.

Pfeiffer and Canon found a specific bacillus in the sputum coughed up in Influenza, and also in the blood.

Morphology. The bacilli occur singly as small rods ·5μ long and ·2μ broad, which rarely unite in short chains. They are non-mobile and do not form spores; are hard to stain by Loeffler's Blue or Carbol-fuchsin, and do not stain by Gram's Method.

Conditions of Growth. The best temperature is that of the blood, and growth will not take place below 28°C. Heat readily kills the bacillus. On Glycerine-Agar tiny semi-transparent colonies appear after 24 hours; but a medium which contains blood seems to serve best for their cultivation.

MEASLES.

Canon has discovered a bacillus in many cases of Measles, which he claims to be the specific cause of the disease. It occurs in the blood and discharges from the eyes and nose, and persists through the whole course of the attack.

Morphology. In form this bacillus appears to be very variable; at times being so small as to resemble a diplococcus; at times much longer. It is extremely hard to stain, a mixture of eosin and methylene-blue in alcohol being necessary to do this in the blood taken from a patient suffering from the disease; but Gram's Method is useless for the purpose. No spore-formation is said to occur in the bacilli, which appear to be mobile.

Conditions of Growth are doubtful. While some declare it can only be cultivated on broth, others say that glycerine-agar yields good results, though either plain agar or gelatine is useless.

Inoculation of the bacilli subcutaneously into mice killed them by a septicæmia.

TYPHUS FEVER.

Levaschew at St. Petersburg has lately found a micrococcus in this disease much resembling Staphylococcus and Streptococcus Pyogenes in form, but differing from them—(*a*) in being much smaller; (*b*) in liquefying gelatine far less quickly than Staphylococcus Pyogenes; (*c*) in producing colonies in gelatine whose characters do not at all resemble those of Streptococcus Pyogenes; (*d*) in not producing any effect after inoculation on animals; (*e*) in being entirely aerobic. This specific micro-organism has been discovered in nearly all cases of the disease,

and although often described before by others, had not been properly isolated. It is found in the blood at the acme of the disease, and also occurs earlier and later, but in such small quantities as very likely to be missed. More are, naturally, found in the blood of the spleen *post mortem* than in that taken from the finger during life: blood from the veins also shews a goodly number.

Morphology. Cocci of ·2μ to ·5μ in size, very refractile, generally occurring singly, sometimes in pairs, clusters, or short chains. Some are non-motile, others very active, and in these a long flagellum can be made out. They stain readily with Fuchsin or Loeffler's blue, but are easily decolorised. The flagellate forms are only found in about 20 per cent. of the cases.

Conditions of Growth. Blood from the veins yields a growth on ordinary media, but that from the spleen only on ascitic fluid gelatine (? because the vitality of the micro-organism is lessened in that viscus). Growth is best at 37°C., but will also take place at ordinary temperatures. The medium must be alkaline; even a faintly acid reaction is fatal. In gelatine stab-cultures the bacilli develop most rapidly on the surface, and by liquefaction of the medium a cylindrical depression results.

Inoculation Experiments on animals have not been successful.

CHAPTER V.

Local Destructive Processes.

These are bacterial diseases accompanied by inflammation at the site of inoculation—a sign that either a large dose of the bacteria has obtained entrance locally, or that they have considerable virulence. The essence of such diseases is that each destructive process is a local reaction, histologically characterised by proliferation of tissue cells with emigration of white blood-corpuscles to the point of attack, and marked clinically by all or most of the four cardinal symptoms—calor, rubor, tumor, dolor. There are various forms of local reactions, such as furuncle, dermatitis, osteomyelitis, buboes, inflammation of mucous and serous membranes, etc., and the occurrence of each implies two things—(*i*) the power of the micro-organism to grow within the organism; and (*ii*) the power of the white blood-corpuscles to migrate and mass at the seat of bacterial attack.

Inflammation.

Various attempts have been made to define this process, but no clear definition from a pathological or bacteriological point of view, except that mentioned below, has yet resulted. The term, however, is a useful one clinically and cannot be given up. Inflammation is not a question of vascular change, for it may occur in animals and in tissues that are bloodless. Metchnikoff describes it as being "the phagocytic reaction of the organism"—which may take place—as in the frog—without any of the recognised clinical symptoms. "Phagocytic reaction is a provision for the protection of the organism against a bacterial invasion"—and this resistance occurs either—(*i*) locally; (*ii*) in the lymphatic channels, or (*iii*) by means of metastatic abscesses.

The inflammation may result in—I. *Cure, i.e.*, destruction of the bacteria at the seat of infection, either through want of food or because the leucocytes destroy them. This is the method of termination of an ordinary boil. If, however, the inflammation be in the lung or other vital organ, such a result might be fatal because the part is vital. II. *Generalisation of the bacteria throughout the body—i.e.*, the bacteria become too powerful for the leucocytes, and spread beyond the site of inoculation.

The methods by which the Bacteria gain access to the blood and thus become generalised throughout the body are—(*i*) By direct invasion of the blood-vessels, as occurs in Tubercle and in Anthrax (malignant pustule); (*ii*) by invasion of the lymphatics, and subsequent dissemination; and this is the more common method. As the bacteria pass from the seat of inoculation along the lymphatic vessels, they reach the lymphatic glands, where they are stopped either temporarily—the gland acting as a sort of difficult filter for them to pass through—or permanently. In a soft chancre the inguinal glands become very swollen, and then suppuration occurs, marking an end of the process. But in a hard chancre these glands are indurated and only slightly swollen: which signifies that they do not act as a sufficient barrier to the progress of the bacteria. Tuberculosis, again, often attacks the Thoracic Duct, and the bacilli then reach the blood-channels—the result being Miliary Tuberculosis.

Fate of the Bacteria after they have gained access to the blood-stream.—(*a*) Development of a Septicæmia may occur, *e.g.*, in Streptococcus infection, by the bacilli outgrowing the leucocytes; or (*b*) a Pyæmia may result, with the formation of metastatic abscesses. Now, the bacteria on entering the blood are taken up by the white blood-corpuscles which lodge them in various organs of the body, where again the same process of infection may take place as happened at the site of inoculation, in other words, metastatic abscesses are formed. (Koch's Pyæmia.) But a Septicæmia may also result, by the bacteria secreting a poison; and indeed it is often impossible, clinically, to distinguish the two processes. Pathologically, however, this can be done; for whereas in a Septicæmia the micro-organisms are found free in the blood at some stage or other of the disease, this is not the case in Pyæmia.

Immunity against Local Destructive Processes in general cannot be said to exist naturally, although different tissues have different susceptibilities to different micro-organisms. It must be noted, however, that in some few cases, it has been practically proved that temporary immunity against local destructive processes is possible. For instance, in a case of gonorrhœa there is probably an interval between two attacks in which the individual is immune—(although one attack of the disease certainly predisposes to another), and again, if one ear of a rabbit be experimentally inoculated with Erysipelas it itself has a period of temporary immunity, although the other ear is not immune, and there is at least only a moderate degree of general immunity.

Four chief varieties of cocci causing Local Destructive Processes concern us. They are very widespread, being found in the dust of rooms, etc. It is probable that in a suppurating wound these micro-organisms undergo a process of selection, and a

virulent form is developed from a harmless variety, and by transmission through different patients becomes still more virulent. The cocci are—Staphylococcus Pyogenes Aureus et Albus ; Streptococcus Pyogenes ; Frænkel's Diplococcus Pneumoniæ; and Neisser's Gonococcus. All these show great difficulty in satisfying Koch's canons for the following reasons: I.—It is often impossible to tell from clinical symptoms to what coccus the particular reaction is due, II.—With the exception of Staphylococcus Pyogenes they are hard to grow, and can only be isolated with difficulty. Further, the virulence of a cultivation rapidly runs down (Staphylococci attenuate more slowly than the others), so confirmatory experiments are difficult to obtain even with susceptible animals. III.—There is often great difference of clinical symptoms of the same disease in man and in animals—(as the most virulent forms are found in man), especially if metastatic abscesses occur.

STAPHYLOCOCCUS PYOGENES AUREUS ET ALBUS.

Staphylococcus Pyogenes Albus resembles Staphylococcus Pyogenes Aureus in most respects, but is less virulent, harder to isolate and cultivate, and shews no pigmentation during its growth. Hence it will be more convenient for us to study its congener, Staphylococcus Pyogenes Aureus, which is more readily obtained —though at first slightly mixed with the other form—by being grown on successive plates of Agar-Agar or Gelatine from an original drop of pus.

Morphology. Non-motile micrococci, ·8μ in diameter, occurring singly, as diplococci, or in irregular masses, and staining with ordinary basic dyes or by Gram's Method. The contiguous surfaces of the cocci are flat, thus differing from the Gonococcus, whose contiguous surfaces are concave.

Conditions of Growth. These cocci will grow either aerobically or anaerobically. On gelatine plate cultivations, circular colonies with smooth round borders are seen within 48 hours, if a temperature of 37°C. be employed, the gelatine being liquefied into small cups, series of which run together and coalesce. As a puncture in a tube of gelatine, growth is rapid, the medium being extensively liquefied and the needle-track presenting a concave surface directed downwards to the bottom of the tube. The growth eventually falls to the bottom as an orange-coloured mass. (Staphylococcus Pyogenes Aureus resembles this growth, but a white layer like varnish is produced, and no coloration). On Agar-Agar and blood-serum, the growth is luxuriant, especially in the latter medium, and when the serum used is obtained from man. The golden colour is only produced in contact with air. No spores are formed under any

circumstances whatsoever. Resistance to dry heat is fairly marked, but moist heat will destroy these cocci in about ten minutes.

Occurrence.—Staphylococcus Pyogenes is met with in acute abscesses ; in suppuration generally; ulcerative endocarditis ; ostemyelitis ; pyæmia, etc. It is present in dust; on the skin ; in the mouth, nose, eyes, and ears of man ; under the nails ; and in human fæces, especially of children.

Koch's Canons.— The first cannot be enforced with regard to the occurrence of these cocci in all cases of the diseases which they are said to produce, but can be enforced with regard to their non-occurrence in tissues apart from these diseases. The second canon is hard to fulfil because the cocci are best grown only on human serum, and even then will attenuate rapidly. The third is satisfied by finding susceptible animals in the rabbit, guinea-pig, mouse, dog, man, etc.

Inoculation Experiments. When pure and virulent, Staphylococcus Pyogenes Aureus is an extremely dangerous micro-organism. When injected subcutaneously in man, it produces severe furnucle locally, and later on spreads by the lymphatics, giving rise to pyæmia. In rabbits, mice, guineapigs, etc., hypodermic inoculation produces an abscess ; but septicæmia follows its injection into the peritoneal cavity or a vein.

Question of Attenuation : *Pfuhl's Experiments.*—Pfuhl found that if he inserted a bit of soldier's uniform under the skin of mice or rabbits—animals which are very susceptible to these Staphylococci —nothing resulted locally ; nor even when the piece of cloth was buried deeply in the muscles. This shewed that the Staphylococci rapidly attenuate in air, which fact it is important to bear in mind with regard to gunshot wounds where bits of clothing are often carried in by the passage of the bullet. The same observer found, however, that if some pus from a human metastatic abscess was first placed on the cloth, and it was then at once introduced hypodermically or into the peritoneal cavity of the animal, septicæmia ensued. This tells us how careful one must be to avoid transferring micro-organisms (especially in a virulent condition) when dealing with wounds.

STREPTOCOCCUS PYOGENES.

This micro-organism, discovered by Rosenbach, is frequently met with in company with the foregoing.

Morphology. Non-motile cocci, ·5–1μ in diameter, arranged in chains or in pairs. No spores are seen. Staining is obtained by Gram's Method or ordinary dyes.

Conditions of Growth. All ordinary culture-media serve. On plate-cultivations punctiform colonies are formed. A temperature of over 24°C. is required. When grown on gelatine no liquefaction of the medium occurs, nor do the colonies tend to run together as they do on serum or Agar-Agar. These cocci can also be grown on milk, which becomes first coagulated and afterwards dissolved ; or even on faintly acid media. It is fairly resistant to drying, but a temperature of 55°C. applied for 15 minutes will be sufficient to kill it, especially if moist.

Occurrence.—In man, Streptococcus Pyogenes is found in abscesses under the skin ; in Pneumonia, Puerperal Endometritis, Erysipelas, and in sore throat resembling Diphtheria. It is seen free in the blood ; which fact, together with its occurrence in Erysipelas, distinguishes it from Staphylococcus Pyogenes.

Inoculation Experiments. Hypodermic inoculation on the rabbit's ear produces a condition resembling Erysipelas, but after inoculation in man, abscesses are formed and the cocci spread by the lymphatics, producing Septicæmia (*cp.* the Streptococcus Septicæmia which often accompanies Enteric Fever).

Anti-Streptococcus Serum, obtained from animals (horses), rendered immune by a process of Mithridatism, has during the past two or three years been used with considerable success in puerperal fevers, septicæmia, acute spreading gangrene, and other cases of Streptococcus infection both local and general.

Koch's Canons are extremely difficult to satisfy, for reasons already explained above.

STREPTOCOCCUS ERYSIPELATIS.

Occurrence.—This coccus, discovered by Fehleisen, is met with in the lymphatics of the skin and mucous membrane in cases of Erysipelas, mostly just beyond the spreading margin of the blush. The cocci probably produce their toxine and then die out, leaving the toxine behind, since no true Erysipelatous cocci are found within the affected tissues themselves. Streptococcus Pyogenes, however, is met with in this situation ; and from the fact of its being able to survive must be less readily killed than the Streptococcus of Erysipelas.

Morphology. Non-motile diplococci, or cocci occurring in chains (the usual form), ·8μ in diameter, not possessing spore-formation, readily destroyed by heat, and staining with ordinary aniline basic dyes or by Gram's method.

Conditions of Growth almost exactly resemble those of Streptococcus Pyogenes. On gelatine small points are seen, without

liquefaction of the medium ; on Agar-Agar and serum the colonies are slightly raised.

Inoculation Experiments. Mice are immune, but the disease can be reproduced from culture-growths by inoculation on the ear of the rabbit, or in the human subject. Very severe results, however, *may* ensue ; and hence it is extremely dangerous to attempt to cure malignant tumours by inoculation with the pure Streptococcus Erysipelatis ; although perhaps, the toxines produced by a very violent growth may be used beneficially, and have been recommended as successful for this purpose, especially if mixed with a sterilised growth of Bacillus Prodigiosus.

NEISSER'S GONOCOCCUS.

Occurrence.—This coccus is seen in the urethral and conjunctival discharges of Gonorrhœa ; on the surfaces of detached epithelial cells from the urethra, and in the cement substance between them. It is met with most frequently and in greatest numbers in the early stages of the disease : later on it becomes very much mixed with contaminating micro-organisms. It is also to be found in the sequelæ of gonorrhœa—endometritis, salpingitis, cystitis, peritonitis, arthritis, etc. The abscesses that occur along the urethra during the course of the disease are due to Staphylococcus Pyogenes rather than to the Gonococcus.

Conditions of Growth are extremely difficult. On human serum at 35° to 37°C. in a moist atmosphere, or on Agar-Agar and serum mixed, it forms, in 18 to 24 hours, small colonies which soon coalesce into a yellow-grey moist layer. Constant transplantation (every 20 to 36 hours) is required to obtain the gonococcus free from contaminating bacteria ; yet by keeping this up, it quickly attenuates. The coccus can also be satisfactorily grown on the liquid removed from ovarian cysts ; and it will survive in acid gelatine or in slightly acid urine. It is not easily killed, and greatly resists drying.

Morphology. Small ovoids arranged in pairs, having no capsule, slightly concave on the surfaces facing each other, which are separated by a narrow biconvex interval. Their diameter varies from $\cdot 8\mu$ to $1\cdot 5\mu$. They are non-motile, possess neither cilia nor flagella, and do not form spores.

Staining Properties.—Aniline dyes generally will serve for the purpose, especially in weak solution, but Gentian-violet or Fuchsin acts best. Gram's method is not efficacious, which fact helps to distinguish the Gonococcus from the Staphylococci, Streptococci, and various diplococci found at times in urethral discharges resembling gonorrhœa.

Re-inoculation Experiments have not yet been satisfactorily carried out on the lower animals. A culture-growth inoculated on the human urethra reproduces the disease typically.

DIPLOCOCCUS PNEUMONIÆ.

Œtiology.—Various micro-organisms have been described in connection with Pneumonia, but Frænkel's diplococcus has been proved to be the specific cause of the disease. This micro-organism can be obtained from the rusty sputum of Pneumonia by carefully washing some freshly coughed-up sputum in distilled water, and transferring a small quantity taken from the centre of the mass to a suitable culture-medium.

Morphology. Immotile diplococci of ovoid form, sometimes occurring in short chains—as when grown in a liquid medium—and surrounded by a thick capsule which greatly resists staining by ordinary aniline dyes, though capable of being penetrated by Gram's method or by Weigert's stain without much difficulty. No spores are formed.

Conditions of Growth. It is hard to cultivate this diplococcus, which only lives for a few days in broth, and gives much trouble in transplanting. Excess of alkali favours its development. On serum a transparent layer is formed by the coalescence of numerous small colonies which first appear on its surface. A temperature of over 24°C. is required, as the micro-organism will not grow below this ; and the best forms are produced at 35° to 37°C. As its growth yields Formic Acid, which probably checks its further development, luxuriance of cultivation is never obtained. Virulence is rapidly lost unless constant transference to a new medium be observed ; and death will result in about 8 days if this be not done. To increase its virulence the diplococcus requires frequent passages through rabbits or other susceptible animals. When grown on Agar-Agar an acapsular variety is produced.

Occurrence.—This micro-organism is found in 90 per cent. of all cases of Pneumonia. It is also met with in Endocarditis, Pleurisy, Peritonitis, and in acute abscesses, *e.g.*, of the outer and middle ear ; and, besides being discoverable in the saliva of a normal man long after he has suffered from Pneumonia, it occurs not unfrequently in the mouths of men who have never had the disease. This illustrates the fact that a number of pathogenic micro-organisms are constantly around, upon, or within us and other animals, which only develop and produce disease under favourable conditions.

Inoculation Experiments do not afford satisfactory phenomena. When animals are susceptible—as mice, guineapigs, rabbits, etc.—they are exceedingly so ; and death occurs, after inoculation, in from a few to 30 hours, a preliminary œdema being rapidly

followed by septicæmia (while the diplococci are at once found around the area of inoculation and in the blood), and the spleen being enlarged and congested. In the dog, an animal which is comparatively immune, a local abscess occurs. The virulence of the diplococci is greatly increased by passage of blood from an infected animal through a series of susceptible ones. In man and in sheep a local reaction only occurs, even by subcutaneous inoculation, which shows that they are not so susceptible.

Immunity in Pneumonia.

Man has a relative immunity from the micro-organism of this disease, as proved by his local reaction ; though once he has suffered from it he appears to be predisposed to further attacks. This suggests the possibility of treatment by means of an antitoxin or other artificial immunising process. Immunity can be produced in rabbits by the injection of small doses of the toxines produced by the diplococci ; but this method is not practicable in the case of man as it is too dangerous. The serum of a man who has recovered from pneumonia is said to confer immunity against the disease on rabbits. In the local lesion phagocytosis occurs, and can be seen in the lungs of croupous pneumonia fairly easily. In pneumonic sputum the leucocytes are found broken down, so that clotting will take place on the addition of pepton-plasma. *Serumtherapy* has lately been used with success in man. To obtain the serum ponies are injected with broth cultivations heated to 60°C. for one hour, and later on with living cultivations either in broth or on Agar-Agar. A definite degree of virulence of the Pneumococcus for this purpose is obtained by a series of 9 passages through rabbits; and the serum obtained from the horse is measured as to its strength by noting the amount necessary to neutralise the fatal dose for an intraperitoneal injection of the standard culture into a rabbit.

Koch's Canons.—The first and third are readily fulfilled, but the second is hard to confirm since growth of the diplococcus is no easy matter.

Friedlander's Bacillus, to which great importance was at one time attached, plays a comparatively unimportant part in this disease. It is stained by aniline dyes, but not by Gram's method. It grows luxuriantly on all culture-media, and is sometimes said to produce gas in gelatine growths. Although pathogenic in dogs and mice, it is not so in rabbits.

BUBONIC PLAGUE OR PEST.

Œtiology.—The term "bubonic" is somewhat misleading. In not a few cases no superficial buboes are seen at all, and these often turn out to be the most rapidly fatal forms of the Septicæmic variety ; while in primary inoculation by the lungs, death may occur before any enlargement even of the nearest lymph glands has had time to occur.

Kitasato described the micro-organism he discovered to be the true cause of Plague as a Cocco-bacillus or Diplo-bacterium but, as a matter of fact, it is a short bacillus of very varying morphology. Its method of invasion of the body is usually by a skin abrasion or by inoculation through a wound (*e.g.*, *post-mortem* wound), though the microbe may possibly be absorbed in the alimentary canal, and most certainly by inhalation into the lungs—but this last method seems rarely to be detected without bacteriological examination. During an epidemic, and especially at its commencement, rats are infected in enormous numbers by Plague, which is essentially a disease propagated by direct social human intercourse, especially under conditions of filth and poverty. A warm moist atmosphere and a high level of subsoil water indirectly predispose by enhancing the conditions for the development of the bacillus.

The micro-organism may be obtained for purposes of investigation from the glands of a patient whose symptoms have reached their height; if attempted too early or too late they may quite likely be missed. This method, though often recommended, is not unattended by danger, for by the pricking of the gland an exit hole is provided in its capsule for the bacilli, which may escape and enter the blood stream direct, thus converting a simple local reaction into a Septicæmia (see further on). The surface of the skin is rendered aseptic, a superficial gland pricked with a grooved needle, and the thick yellowish white lymph it contains squeezed out: blood should be avoided. For culture-purposes it is better to obtain the bacilli post mortem from the spleen, blood, glands, or viscera of a Septicæmic case—a very easy method. Streptococci may be found as a secondary infection in some instances, but the typical Plague bacillus is found in all cases of the disease.

Morphology. A short thick bacillus, about one-sixth the diameter of a red blood-corpuscle in length, and but little more than half as much as that in breadth; having its ends rounded and a small spot, which refuses stain, in its centre. This spot varies greatly in shape, position, and size; sometimes it is ovoid or circular, the protoplasm of the bacillus completely surrounding it; sometimes it is like a transverse band—the stained part of the bacillus being at the poles; sometimes it is lateral; in other instances it occupies nearly one-half of the bacillus from the centre to the pole; or it may be even multiple, simulating pseudospores. The bacilli occur singly, in pairs, or in short chains; but when grown on artificial media, and especially in broth, longer chains may be found. They are non-motile, and can be readily killed in 3 or 4 hours by direct sunlight, by moist heat at 65°C. for 40 minutes, or by weak antiseptics. If spread out on glass plates drying—in daylight—kills them in 20 hours or so, and they die in 48 hours if the plates be kept in the dark; on woollen material they die after 4 or 5 days,

on linen in 3 days. A virulent culture on Agar-Agar is destroyed if kept in strongly diffused daylight for 40 to 48 hours. Ordinary basic dyes serve well as stains—Carbol-fuchsin being perhaps the best; but Gram's method is of no avail. In tissues and occasionally when taken from cultures, the bacillus often appears to resemble a large diplococcus in form, but can be distinguished by clearing the specimen. As seen in nature it possesses a well-marked capsule, but loses this in time by cultivation on Agar-Agar and almost at once on gelatine. In old Agar-cultures or when the media are insufficient or not quite suitable (through being acid, neutral, too dry, too alkaline, etc.) peculiar forms are developed, *viz.*, elongated bacilli many times as long as the typical variety; pear-shaped and club-shaped forms, swollen to 6 to 12 times the size of the original, which take stain very badly; dumb-bell shapes of different sizes; large circular forms occurring singly or in short chains, etc. These are known as *Involution Forms*, and may be found in the glands post mortem or in the pus of a freshly opened suppurating bubo. They usually shew either no unstained portion at all, or only very indistinctly. By cultivation on new and suitable media the original forms may sometimes be re-attained, but often this is impossible without first passing the bacillus through a highly susceptible animal.

Conditions of Growth.

All ordinary media will serve, but ordinary Agar-Agar rendered only just alkaline is the most convenient and best; Glycerine-Agar with 2 per cent. Peptone and 2 per cent. Gelatine, or Serum-Agar, serve better than ordinary serum. On Agar-Agar plates at 37°C. in 40 hours small characteristic greyish-white circular glistening colonies appear. They are separate, do not run together, and *en masse* give the appearance of ground glass slightly polished; in reflected light they have a bluish appearance. These characteristics may even be distinct at the end of 24 to 30 hours, and are seen on Agar-slant tubes also. Each colony is irregularly circular in outline, but well defined, more granular in the centre, which appears darker, than the periphery. No liquefaction of the medium occurs in any instance. On serum growth is rapid, but the bacilli remain quite small, appear to lose their cross-bands, and rarely swell up into involution forms. On gelatine plates and slant-cultures the colonies appear more circular, very well defined at their margins, and distinctly granular on the surface; the bacilli are small, but preserve their cross-band which remains well-marked; and no swelling occurs. In Gelatine punctures fine granulations run vertically downwards in strings (much like Fowl Cholera); no liquefaction takes place. Rapid growth is observed in Bouillon, a characteristic cloud first appearing in suspension with the supernatant liquid remaining clear, and after a few days the bacilli adhere in chains and fall down to the bottom of the tube or flask as thin whitish, yellow flakes. If

small particles of fat be placed on the surface of the bouillon, fine, wavy, hair-like festoons of the bacilli in chains grow downwards from them in 2 or 3 days into the medium, which must be kept perfectly still, without being in the least disturbed, for a considerable time to obtain a thoroughly characteristic appearance. Growth in all media is distinctly more rapid in the dark, and the virulence of the bacillus becomes greater. On Agar-Agar some colonies appear much bigger than others, and can often be perpetuated as such in other tubes, but usually return to the usual form on further transplantation. Certain shapes obtained in the course of growth can also be occasionally carried on from tube to tube in Agar and the original characteristic form only regained after passages through animals. The colonies on Agar and Serum can be moved bodily along the surface of the medium by a platinum wire, but when touched on their own surface adhere very tenaciously, and can be drawn out as an opaque sticky thread. On Gelatine this is not nearly so characteristic, and the colonies themselves are somewhat adherent to the medium. On artificial media virulence is rapidly lost unless passages through susceptible animals are kept up; and transplantation is necessary at least once a month.

Inoculation Experiments. Susceptible animals have been discovered in man, mice, rats, guineapigs, rabbits, and other small rodents; monkeys are also susceptible, but horses, cattle, dogs, cats, pigs, and birds are apparently not. The bacilli described in connection with these animals dying during a plague outbreak are really due to their own special diseases, *e.g.*, fowls shew bacilli of fowl cholera (much smaller than though resembling Pest bacilli) and pigs swine fever, etc. An inoculated mouse in 24 to 36 hours appears dull, and suffers from debility, anorexia, very rapid respiration, and a purulent conjunctivitis; it becomes lethargic, lies on its belly, with its fore and hind legs often stretched out and not doubled up under it; convulsions may surpervene before death, which usually occurs in about three days after inoculation at the root of the tail. If the bacilli are very virulent, or a large quantity be injected under the skin, death may occur even as rapidly as in twenty-four hours. Post mortem the spleen, lungs, liver, and sometimes the lymphatic glands are found congested and enlarged; pleural and peritoneal effusions may be present, and also local œdema at the seat of inoculation. The blood, spleen and internal organs shew crowds of Pest bacilli.

Immunity. This is possible in the horse, but has not yet been proved to have been artificially produced in man, although Haffkine's method may bring about such a result.

Yersin, working in Saigon, reported that he had discovered a bactericidal and antitoxic serum, which was prepared by injecting pure Pest bacilli, in successive and increasingly large doses, into a horse, until the animal could stand an enormous quantity, when its serum was found to be as described. Much time is required in its preparation, as the injections can only be given about once a month at first. Very favourable results were reported in a small number of cases in Amoy; but in Bombay the results obtained by Dr. Yersin with the serum he prepared himself in the above way have not been nearly so encouraging as was generally anticipated. Enormous quantities frequently have had to be given—often over 200 c.c. altogether—and even then without any appreciable effect on the course of the disease. It appears to be of little use unless given at the very commencement of the attack, and quite useless in severe cases. Some few mild cases, however, have been distinctly benefitted. Most distressing urticaria, and synovitic affections lasting weeks, not rarely follow the injection of even ordinary doses. Another serum, obtained by using dead Pest cultures for injecting into ponies, which has lately been received from the Pasteur Institute, is reported to be no better than the former ; and a third, said to be highly antitoxic, which Professor Roux is now preparing in Paris, is eagerly looked forward to as likely to uphold the reputation of results achieved in Amoy.

Haffkine, in Bombay, found that by adding a weak solution of carbolic acid or essence of mustard to a virulent growth of Pest bacilli in bouillon or other media, or by acting on such a growth by heat (70°C. for one hour, or 65°C. for several hours) the micro-organisms themselves are destroyed, but their products which remain possess a great protective power when inoculated into animals against the disease. He is, therefore, making extensive experiments in mankind for this purpose, and so far his results appear most favourable. The vaccine, however, seems difficult to maintain at a continuous value ; there does not appear to be any standardised dosage ; nor has it yet been scientifically proved to produce a bactericidal effect in the serum of the organism inoculated, though it is to be hoped and seems not unlikely that both its bactericidal and antitoxic value for mankind will be definitely ascertained later on.

Course of the Disease in Man.

Clinically, we note the sudden onset with high fever, sometimes preceded by rigors, malaise, and prostration, and followed rapidly by headache, which is generally frontal, and often by delirium: while restless efforts at vomiting which may or may not be successful often occur early in the attack. The formation of buboes may precede, but generally follows the rigor : superficially, the femoral glands most often and next in frequency those in the axillæ and neck are found to be enlarged and exquisitely tender ; and the position of the primary bubo affords a direct guidance to the seat

of inoculation, which can often be seen and the development of some local lesion made out. Constipation is the rule, but diarrhœa may occur, slight diarrhœa being probably a good sign. Sloughing sores, carbuncles, and symptoms due to infarctions in various organs may complicate the course of the disease. Death occurs in from a few to sixty hours in bad cases, but in those which pass on to recovery the disease lasts longer. If a patient survives one week the prognosis depends on his constitutional powers of repair rather than on the disease itself.

Pathology. The Pest bacillus, as we have seen, gains an entrance into the body either—(*i*) by inoculation through an abrasion or wound of the skin or mucous membrane ; (*ii*) by inhalation into the lungs ; and (*iii*) by absorption from the alimentary canal. When invasion is by the first method the bacilli may produce a local lesion at the point of entrance, *e.g.*, a vesicle, but usually most of them pass rapidly up the lymph-vessels to the nearest set or sets of lymphatic glands which enlarge and become congested, and may arrest their progress completely (Simple Local Reaction tending towards recovery—and if other glands become enlarged at this stage, it is due to absorption of toxin with or without the presence of dead bacilli) ; or they may get into the blood—(*a*) by passing further along the lymphatic chain, reaching the Thoracic Duct, and thus eventually the left Subclavian Vein ; or (*b*) by direct hæmorrhagic infiltration spreading through the vein walls into the lumen of the vessels from the affected glands lying in direct contact with them. (In these two ways we get the fatal Septicæmic forms, simple or hæmorrhagic, of the disease.) By the second method the local process in the lungs may end fatally *per se*, or by causing a Septicæmic process by general extension, with or without glandular enlargements. The bacilli in this case will be found in the sputum from the first, and this is useful to note in order to distinguish this primary lung affection from without from the secondary invasion of the lung by Pest bacilli from within, that occurs by infarction and hæmorrhage during the course of the Septicæmic variety—the bacilli being found later and the sputum being characteristic of any complicating lung disease that may exist. The third method has not yet been conclusively proved, and possibly is more often due to a local intestinal inoculation of an abraded mucous membrane than to absorption by a healthy one. Thus, to summarise, inoculation is followed by partial local reaction and this by generalisation of the bacilli throughout the body: or the reaction may remain more or less local; but an acute form occurs, in which no local reaction may be found ante or post mortem, and is invariably fatal. If the bacilli are found in the blood by bacteriological examination, the Septicæmic stage has been reached, and the prognosis is invariably bad ; they are never found

in the blood in cases which terminate in recovery. In man the glands often suppurate if recovery takes place (though living bacilli are not always found in the pus), thus marking a successful attempt at local resistance: on the other hand, the bacilli may die within them and the whole undergo complete resolution and absorption. Re-infection appears possible; and cases undoubtedly do occur in which a second attack, often fatal, follows a mild first dose of the disease.

TUBERCULOSIS.

Distribution.—This disease is most widespread and has persisted from the earliest ages. In the human race both sexes, young and old, civilised and savage, suffer; while in Europe alone about 20 per cent. of mankind die of the disease, which also attacks many animals—especially cattle, goats, pigs, and captive monkeys. It is rarely found amongst sheep, horses, cats, and dogs; and occurs in a modified form in birds and reptiles.

Stages in the History of the Pathology of Tuberculosis. *I.*—Bruisser used to teach that Tuberculosis was a slow inflammation of the lungs—a variety of pneumonic or other inflammation, in fact. *II.*—But Lænnec denied this view, and looked upon the whole lung as being infected by tubercular inflammation, the only important lesions, however, being the caseated masses found post mortem. In other words, he regarded it as a dual process of—(*a*) Caseating Tubercle; and (*b*) Tubercular inflammation—a sort of pneumonic process. By "tubercle" he, of course, meant a small mass, without reference to the bacterial origin of the disease. *III.*—Virchow next said—(*i*) that the "tubercles" or small hard masses found in the lungs (now known as the earlier stage) were the essential lesion; and—(*ii*) that the large caseations were pneumonic lesions referable to the inspissation of non-tubercular inflammatory products. *IV.*—Baumgarten held the identity of the granular ("tubercular") and pneumonic processes, but regarded the "tubercles" as miliary microscopic patches of pneumonia. *V.*—Koch found bacilli, as also did Baumgarten, but neither he nor the latter could stain them properly for identification purposes. *VI.*—Cohnheim showed that infectivity of Tuberculosis existed by inoculating the external chamber of the eye in rabbits with tubercle. *VII.*—Koch at last found that by adding an alkali to methylene blue, he could manage to stain the bacillus properly. Ehrlich subsequently used Gentian-violet and Scheele Carbol-fuchsin; and the bacillus was found not to part with its stain on treatment with an acid. *VIII.*—In the pathological anatomy of Tuberculosis nowadays—(*a*) Lupus is regarded as a process dependent on the tubercle bacillus; and (*b*) the catarrhal pneumonia of phthisis (Lænnec's infiltration) also.

Morphology. Delicate single non-motile rods with rounded ends, without cilia or flagella, slightly curved, occurring in pairs with adjacent ends approximating at an angle and often slightly overlapping, but never touching; very rarely seen as short threads. The bacillus measures 1·5 to 3μ long and ·2 to ·5μ broad, being usually described as having a length equal to half the diameter of a red blood-corpuscle. Small clear spaces are sometimes seen in the middle of the bacillus, giving it a beaded appearance. These are Pseudospores—not true spores—as they are multiple, do not always occur even under the same conditions, and do not take stains—for the simple reason that there is nothing there to stain.

Staining Reactions.—Though rather difficult to stain, the tubercle bacillus is very tenacious of the colour it takes up and will not part with it even to fairly strong mineral acids. When required to be stained in the sputum, a small mass of the greyish-yellow globulated expectoration should be obtained, washed carefully in distilled water, and a small portion transferred from the centre of the mass to a coverglass on which it is spread and fixed by heat. *I.—Ehrlich's Method.*—The coverglass is placed face downwards on the surface of Gentian-violet solution (*see* Chapter X.) and kept thus for 24 hours at 37°C. in an incubator. It is then lightly washed, first momentarily in distilled water, then for 30 seconds in 25 per cent. Nitric Acid, and lastly in water and then Rectified Spirit until the colour is almost lost. A counterstain (*e.g.*, Bismarck-brown) may be used to colour epithelial cells, pus-corpuscles, etc. *II.*—For more rapid results the *Ziehl-Neelsen method* is more convenient. This consists in placing a drop or two of carbol-fuchsin solution on the coverslip on which the sputum has been lightly spread and dried; heating over a spirit-flame until steam is just seen to come off; washing off excess of colour (except from the bacilli) by 30 to 40 seconds' immersion in 25 per cent. Sulphuric Acid; washing in distilled water to get rid of the acid; and counterstaining leucocytes, etc., by Loeffler's blue. Washing, drying, dehydrating, and mounting in Canada Balsam complete the process. *III.—For staining in tissues*, Ehrlich's method may be tried, the sections being left for 24 hours in the stain, or *Unna's method* may be used instead. In this latter, Ehrlich's stain is used at first, and then the section is well washed in water for 10 minutes, after which it is left for 2 minutes in 20 per cent. Nitric Acid which turns it green-black. On being transferred to and gently moved in absolute alcohol, the blue colour returns. The section is next washed in distilled water, placed on a slide, dried with filter-paper, and the slide heated over a spirit flame until the section (which lies uppermost) looks shining, when it is mounted in Balsam. *IV.*—Gram's Method may also be used

successfully, but as it stains so many other bacilli also, it is of not much use in the case of Tubercle.

Occurrence.—The tubercle bacillus may be found in the lungs, sputum, lymphatic glands, spleen, kidney, bones (caries), in cases of Tuberculosis; and is also met with in Lupus, etc.

Conditions of Growth. Being purely parasitic, the Tubercle Bacillus can only flourish properly on the bodies of higher organisms ; although it can be cultivated artificially on culture-media, *e.g.*, broth, gelatine, and serum. It will only grow on Agar-Agar if 5 per cent. Glycerine has been added, the colonies appearing at the end of two weeks as grey-white scales. To obtain artificial growth, either tubes must be inoculated from the centre of a nummulated mass of sputum which has previously been carefully washed in distilled water ; or from the caseating glands of a guineapig inoculated two months previously with tuberculous material. However grown, the bacillus multiplies and develops slowly, and requires a temperature nearly that of the body, *viz.*, 37°C.—any increase or decrease being unfavourable. Temperatures above 70°C. soon kill it. It is both aerobic and anaerobic : possesses great resistance to desiccation, and can be kept in the dry state for years without losing its virulence. When grown in a culture-medium, light should be excluded from it, to preserve its virulence. Antiseptics do not destroy it so readily as might be expected, although for this purpose Carbolic Acid appears to be more efficient than Perchloride of Mercury.

Koch's Canons. I.—The difficulty of finding the bacillus and staining it has been practically overcome in all cases. *II.*—That of excluding adventitious bacilli and obtaining a pure cultivation was at length mastered by using tubes of sterilised serum—as it is hard to get the bacillus pure in plate cultivations. Koch managed this, as has already been mentioned, by first infecting guineapigs and then inoculating tubes ; and so carrying on from one generation of the bacillus to another. *III.*—Susceptible animals are readily found in the guineapig, rabbit, etc. In fact, nearly all domesticated animals are susceptible.

Question of Infection. As the tubercle bacillus has no saprophytic existence outside the body, infection takes place from animal to animal ; and this may occur either by the bacillus being breathed in, swallowed, or gaining an entrance into the body through a wound or abrasion of the skin or mucous membrane. In the last method it is possible that the disease may be inoculated during coitus, *e.g.*, from primary affection of the testis in the male to the female. Further, a few cases are on record in which the disease is credited with having been transferred from the mother to the fœtus through the placenta ; but this is extremely doubtful.

Chief means of dissemination as regards man.

I.—By milk, especially from cows suffering from tubercular inflammation of the mammary gland. In fact, one may say, that a child during its first year of life does not become infected with tubercle from its mother, but from cow's milk; and it has been shown that pigs and cats can easily be made to take tubercle bacilli by means of infected milk—(see also experiments on cattle, further on). *II.—By meat*, especially if underdone—though there is less chance of infection by this means since an attempt at cooking is always made—obtained from a tuberculous animal. The Jews are most circumspect about this, and inspect animals set apart for food first before they kill them for their own consumption. *III.—By Sputum*, and other tuberculous discharges, especially when inspissated and dried, for this gets blown about a room or a hospital ward and can easily infect persons with a "tendency," who appear to be perfectly sound. Cornet mapped out various regions round the beds of tubercular patients, and inoculated guineapigs with dust taken from these different zones. He found that death occurred most rapidly in that guineapig which had been inoculated with dust taken from the zone nearest the patient, and was delayed more and more the farther away from the patient the zone whence the dust was taken.

Inoculation Experiments.—When a guineapig is hypodermically inoculated with tubercle bacilli, nothing occurs for about a fortnight, but then the animal begins to lose appetite and weight. A nodule may be felt at the point of injection, and the nearest lymphatic glands become enlarged. The atrophy continues, hectic fever sets in, and death occurs in about 12 to 15 weeks; post-mortem examination revealing "tubercles" at the site of inoculation, in the neighbouring glands, in most of the other glands, in the lungs, liver, kidneys, peritoneum, etc., with here and there the process in a necrotic stage.

The course of the disease after inoculation may be divided into three stages. *I.*—Local resistance first occurs, but this stage may escape notice. *II.*—Resistance in the lymphatics, as shewn by the appearance of Lupus, glandular swellings, etc., in man. *III.*—Invasion of the blood-stream and development of a pyæmia—*i.e.*, Miliary Tuberculosis, as that occurring in the lung of catarrhal pneumonia.

Intimate nature of the process which produces the morbid lesions.—This was shewn by Metchnikoff, who watched the effect of injection of tubercle bacilli directly into the blood-stream.—(*I*) Disappearance from the blood of both tubercle bacilli and polynuclear leucocytes, to the lungs chiefly, and to a lesser extent the spleen, liver, and other organs, where phagocytosis of the bacilli by the polynuclear leucocytes occurs, to the destruction of the latter in the end; (*II*) two or three days afterwards no polynuclear leucocytes are found, but a great number of large

mononuclear leucocytes are present instead, and can be seen to contain bacilli and bits of the broken-down polynuclear leucocytes; (*III*) several of these large mononuclear leucocytes fuse together and form what is known as a "giant-cell"—this occurs in various places; (*IV*) supervention of (*a*) largely nucleated epithelioid cells, which are found free around the giant-cells and also contained in the meshwork of their branching processes, and (*b*) small mononuclear leucocytes (lymphocytes) outside these; and (*V*) the conflict between the bacilli and the leucocytes either results in—(*a*) the bacilli being worsted in the giant-cell and becoming encapsuled by a deposit of Calcium Salts, or (*b*) the bacilli getting the better of the struggle and, by the poison they produce, causing the death of the giant-cells—caseation following round the spot. What usually occurs is this: The giant-cell dies and the bacilli become diffused by the lymphatics which run alongside the blood-vessels (producing Tubercular Peri-arteritis) and bronchi (Tubercular Peri-bronchitis). The inflammation caused by the bacilli may spread into the intimate lymphatics of the lung and so reach the alveoli, whereby a pneumonic process is set up, catarrhal in nature. Now the cells which fill up the alveoli in Croupous Pneumonia are leucocytes; and in Tubercular Pneumonia the cells in the alveoli must be either leucocytes or due to the proliferation of the endothelial cells lining the alveolar walls. Metchnikoff found, in experimenting with the air-bladders of fishes, that their endothelial cells have no phagocytic action. He argued, therefore, that those in the alveoli of the human lung do not possess phagocytic property, either. But the cells which are found in the lung alveoli in the catarrhal pneumonia of Tuberculosis contain bacilli, showing that they do possess phagocytic action. Hence, they are not alveolar endothelial cells, but leucocytes. They are capable of taking up dust-particles ("dust-cells" of the Germans) and are, in all probability, only mononuclear leucocytes—for the polynuclear have disappeared. It was objected that, since leucocytes are found in Croupous Pneumonia also, in what way does that variety differ from the Catarrhal form? The answer is given in the fact that in Croupous Pneumonia the cells are polynuclear leucocytes and fibrin is found in the alveolus; but in the Catarrhal Pneumonia under discussion the leucocytes are mononuclear. Thus, the difference between the two processes is only really a difference of leucocytes entering therein; and indeed it is stated (Ehrlich) that the blood in Tuberculosis may be recognised by the fact that it contains no polynuclear leucocytes.

If the formation of "tubercles" is a matter of phagocytosis, then they ought to be found independently of the Tubercle-bacillus. This happens to be actually the case, as they are formed if the round-cells of Nematodes are injected into cats and dogs. Dead Tubercle-bacilli on injection, will also cause the formation of

"tubercles"; but such tubercles do not grow and only last a short time; whilst living active Tubercle-bacilli will cause a "tubercle" to increase in size.

The effect of a previous attack in producing immunity in Tuberculosis.—Just as immunity does not appear readily possible in other local destructive processes, so would it seem to be the case in Tuberculosis. Tubercle under the skin, or in the lungs or larynx, is certainly a local destructive lesion, but the process is so very chronic that it is impossible to judge of its immunising power. The question resolves itself into whether Tubercle kills by a local process or by general poisoning. The examples above-mentioned shew that it can kill by local action, and it was by such reasoning that Koch was led to the consideration of his "*Tuberculin.*" He found that by hypodermically inoculating a tubercular guineapig with Tubercle-bacilli the local process becomes far more severe at the expense of the general process, local gangrene and necrosis occurring. In fact, it is far more so than if a healthy guineapig be primarily inoculated, though the lymphatic glands do not become affected for the reason that the secondarily inoculated animal already has tubercle in its system. Koch further noted that the same result was obtained when, instead of the bacilli, he used pure cultures of the dead bacilli for the secondary inoculation—considerably prolonging the life of the primarily inoculated guineapig in both cases. On extending his observations, he found that a 50 per cent. Glycerine extract of cultures of the Tubercle bacillus acted similarly to the dead cultures he first used. This substance he called "*Tuberculin,*" which is prepared as follows:—Tubercle-bacilli are allowed to grow in broth to which 5 per cent. Glycerine has been previously added, and the whole then sterilised at 37°C. for 6 weeks. By that time the bacilli have died, but their products remain, and this mass is concentrated to one-tenth its bulk by boiling. Koch argued that if he inoculated a normal guineapig with Tuberculin first, it would, on tubercle bacilli being subsequently injected, react like the tubercular guineapig above described; a local reaction would result instead of a general infection. As a matter of fact, however, it was later found that the normal guineapig could tolerate Tuberculin far better than the tubercular one, being able to take a much larger dose; and on extending these observations to mankind similar results were generally yielded, although in many cases of Lupus after an intense local reaction the disease appeared to heal, but returned eventually at the margin of the patch in the majority of cases. Tuberculin, however, does not affect the bacillus itself directly, but by causing excessive necrosis of the tubercular tissue cells at the margin of a tuberculous mass hastens their absorption, and thus affects indirectly the formerly quiescent tubercle bacilli which are partially absorbed also; and by the setting free of a material which they had themselves formed affords them a medium by whose means they can be generally

diffused throughout the system. A healthy man, it is stated, can take as much as $\frac{1}{10}$ c.c. of Tuberculin by injection, whereas a tubercular subject often cannot tolerate even $\frac{1}{1000}$ c.c. By the disastrous and unfortunate results that followed on the too enthusiastic trial given to Tuberculin on man, and its subsequent overthrow as a method of treatment, much opposition has been set afoot against similar scientific cures or protective measures in other bacterial diseases. It is possible, however, that the histolytic action of Tuberculin itself may lead to good results with respect to mankind, such as in the production of an "Anti-tuberculin."

Heredity of Tubercle.—Since those newborn children who have been inoculated with Tuberculin do not react to it, it probably establishes as a fact the general belief that no one is actually born with tubercle in his system, although the tendency to its development may exist most strongly even in the fœtus. Tubercle bacilli have never yet been found in the infant at the time of its birth, and are probably—in spite of the difficulties that may exist for their demonstration—not present.

Prophylaxis against Tubercle; *Value of Tuberculin.*—Experiments on cattle, conducted in Germany, resulted in the discovery of the fact that many animals, certified by Veterinary Surgeons to be in good health and free from disease, were found to be tubercular, as they all gave reactions and many died after small doses of tuberculin had been injected into them. Since such reaction can be obtained, it would probably be best if all cattle were so treated, in order that all tubercular ones might be separated and never used for milking purposes or for food; for although somewhat an expensive process, it would be an extremely sound basis to start upon for the prevention of Tuberculosis, and much of the disease at present existing would be put an end to. On man, the dangers attending the use of Tuberculin directly more than counterbalance its value at present, since it is often most difficult or impossible to accurately determine how far the tubercular process has gone within the system before it is injected. But even in the human subject it would appear occasionally to be of use, as in some cases of Lupus; and Dr. Sandberg, of Bergen, states that he has found Tuberculin to be of distinct value therapeutically in cases of tuberculous joints and tuberculous epididymitis. Indirectly, however, since man usually becomes tubercular through milk in the earliest years of his life, or by meat at any other age, the value of Tuberculin with regard to the human race might certainly be made very great.

An Anti-tuberculous Serum has somewhat recently been brought forward by Professor Maragliano, and is accredited with marked antitoxic power in the treatment of the disease.

After numerous attempts to improve Tuberculin, Koch has at last quite recently succeeded. Three new "*Tuberculins*" are

described, A, O, and R. *Tuberculin A*, obtained by extraction from Tubercle bacilli by one-tenth normal soda solution, reacts like old Tuberculin, but more intensely. *Tuberculin O and R* are obtained by first well pounding dried cultures of Tubercle in a mortar and then adding distilled sterile water. The mixture is next centrifugalised and the clear liquid, which collects at the top and contains no tubercle bacilli, is Tuberculin O. The remainder, constituting Tuberculin R, is dried, pounded, distilled water again added, and the whole centrifugalised for a second time. This process is repeated until hardly any residue remains. To preserve them 20 per cent. of glycerine is added to both preparations. Tuberculin R, alone is recommended for clinical use. Its dose is ·002 mgrm. gradually increased up to 20 mgrms. No reaction occurs ; no ill-effects have been noticed : and the results appear most encouraging. Tuberculin R, like most other remedies, is only of use in the early stages of the disease.

LEPROSY.

Distribution.—This bacillus, which was discovered by Hansen, is found in crowds in the leprous nodules under the skin in cases of the disease, and inside nerves, but not in the internal organs. The tissue of the node is hard, and no caseation is seen, as occurs in Tubercle.

Morphology. Leprosy bacilli are much like Tubercle bacilli in form and staining properties, but are if anything somewhat shorter, more constant in size, with pointed ends, and not so curved. They are not scattered about, but are seen in dense crowds in the tissues in which they are found. Ordinary aniline dyes or Gram's method or Weigert's fibrin stain will serve to stain the bacilli, which frequently present an appearance of pseudospores, like Tubercle bacilli.

Conditions of Growth. No culture-medium seems to have been discovered, through Bordoni claims to have grown Leprosy bacilli on a mixture of human blood serum and glycerine, but as the bacilli he cultivated were morphologically unlike those seen in the tissues, the fact of his having obtained a true growth of leprosy bacilli seems to be somewhat doubtful.

Infectivity. Ortmann is said to have inoculated the anterior chamber of a rabbit's eye with a piece from a fresh leprous nodule, and obtained the bacillus afterwards from new formations in all the internal organs of the animal. This has, however, been denied by many, who declare that it was not Leprosy, but Tuberculosis, that was inoculated. It has also been stated that Leprosy was produced, after

the lapse of some years, by the inoculation of a piece of fresh leprous tissue subcutaneously in a condemned criminal. Monkeys have been experimented upon, but no definite results obtained; so although the infectivity of Leprosy is extremely probable, it cannot be said to have been definitely proved as yet, unless we accept the one case of the criminal above mentioned as conclusive. As the disease is not readily contagious even in leprous districts, it probably gains entrance to the system through a skin abrasion and not by the digestive and respiratory tracts, like Tuberculosis. It is hereditary, like Syphilis, and appears to be spread by the sole agency of man to man.

GLANDERS.

Morphology. Discovered by Loeffler, the bacillus of Glanders is difficult of identification, as it is found mixed with so many others. It is a non-motile bacillus, shorter and thicker than the Tubercle bacillus which it resembles greatly; possesses both spores and pseudospores, and is very resistant to drying. It is extremely difficult to stain, since it both takes up and parts with its stain readily. A good procedure is to stain a section containing the bacilli for half-a-minute in a saturated solution of Methylene Blue in 5 per cent. Carbolic Acid; wash in water and then in 10 per cent. Tannic Acid solution for nearly one minute; counterstain with Eosine, and again wash in water; dehydrate and mount.—The bacilli are blue in a pink ground. Gram's Method is ineffectual.

Conditions of Growth. All ordinary media will serve for its artificial cultivation, but the bacillus rapidly attenuates. On potato, between 25°C. and 40°C., it first appears as a faint yellow mass which later assumes a characteristic brown colour. Gelatine cannot be used for its cultivation, because of the temperature required; but Agar-Agar—especially if Glycerine has been added—or serum, will serve very well. The bacilli may be obtained, for artificial growth, straight from the caseating purulent nodes seen in cases of the disease, or better still by first inoculating some of this material into a male guineapig and then obtaining the bacilli from the pus and exudation fluid in the Tunica Vaginalis Testis of that animal about 3 weeks later.

Susceptible Animals.—Horses, asses, cats, and the larger Felidæ, and dogs, are susceptible, as well as man. Field-mice are extremely so, while house-mice are immune; and cattle, rats, and white mice enjoy a similar privilege of immunity. Guineapigs are very easily infected, but rabbits appear to be moderately resistant.

Inoculation Experiments. After subcutaneous inoculation in the guinea-pig—(*i*) local reaction occurs first, in 4 or 5 days, in the form of swelling at the point of injection, and a node forms which later on becomes caseous and ruptures—producing ulceration of the skin (which may heal); and this is followed by swelling and suppuration of the lymphatic glands, due to emigration of the polynuclear leucocytes containing the bacilli; and (*ii*) in 3 to 4½ weeks the disease generalises into a pyæmia; the testicles and joints enlarge and suppurate, while so-called "tubercles" are to be found post mortem in the liver, spleen, kidneys, and lungs. In field-mice death occurs in 2 or 3 days without any previous local manifestation.

The clinical features in the horse are marked by four forms, *viz.*—(*i*) a nasal variety of the disease in which the Schneiderian membrane becomes affected and "sores" occur in the nose; (*ii*) a pneumonia may follow on the foregoing or take place by itself; (*iii*) "Worm-bugs" or "Farcy" may form in the subcutaneous tissue; and (*iv*) an exanthematous pustular rash may occur.

The clinical features in man are due to infection through the subcutaneous tissues followed by a general disease.

Immunity. White rats, which are naturally immune, may be made to take the disease by administering Phloridzin to them first and causing a glycosuria, after which they become readily amenable to inoculation.

Mallein.—This is a substance allied to Tuberculin, and is prepared from the bacillus of Glanders in exactly the same way as Tuberculin is obtained from the Tubercle bacillus. It is of great use for diagnostic purposes, but of no avail in the production of artificial immunity. If more than 1·5°C. rise of temperature follows the injection of a given dose of Mallein, this fact is said to be pathognomonic that the animal in question is suffering from Glanders.

SYPHILIS.

Lustgarten has discovered a bacillus in syphilitic tissues which he claims to be the specific cause of the disease; but Koch's canons are by no means fully satisfied by it.

Morphology. An S-shaped bacillus, found within large ovoid cells—each cell containing one or two—measuring 4·5μ long and ·2μ broad, and occasionally appearing swollen at the ends. Spores or pseudospores are to be seen within its protoplasm. In order to stain it, sections of the tissue are left for 24 hours in Gentian-violet solution at ordinary temperatures; washed in alcohol; passed through 1·5 per

cent. solution of Permanganate of Potassium, after which they are rapidly passed through weak Sulphurous Acid; washed, dehydrated, and mounted. The bacillus of Syphilis does not part with its stain so readily as do those of Leprosy, Glanders, and Tubercle.

Growth. Artificial cultivation of this bacillus has not yet been attained.

Inoculation Experiments. Inoculation is readily performed on mankind, and possibly also on some of the higher apes, but in no other animals are symptoms typical of the disease reproduced.

Serum-inoculation Treatment.—The serum of dogs, goats, and calves, which have previously been inoculated with serum or matter taken from sores or condylomata of patients in the secondary stage of Syphilis, has lately been found to act most beneficially on syphilitic chancres, rashes, etc., when injected into man—several such cases having been reported. The process of Syphilis is quite comparable to those diseases aforementioned whose bacilli its own resembles. First comes the local reaction, then presumably the micro-organism gets carried into the blood and a general rash follows, and finally, the gummatous patches are found in the internal organs—a sort of pyæmic process. In the syphilitic fœtus the liver is found full of "tubercles" which have been formed, probably, by the syphilitic micro-organism.

Van Niessen's Syphilococcus. Van Niessen, in Vienna, states that he has found a specific micro-organism of the nature of a coccus in an excised primary sore of the prepuce, in gummata and in syphilitic lesions of the brain and spinal cord. Cultivations were made by imbedding small pieces of spinal cord and primary sore in gelatine and colonies soon developed. The coccus can also be grown on bouillon. Van Niessen injected three rabbits, a goat, a guineapig, and a pigeon with such a broth culture, and in several animals a hard sore developed, and in the goat and one rabbit gummata formed later. His experiments have given rise to much discussion, but he claims to have discovered the true micro-organism of this disease, although he has not been able to reproduce typical hard chancres on the penes of animals.

CHAPTER VI.

Intoxication Processes.

In diseases classed as Intoxications, *e.g.*, Diphtheria and Tetanus, two chief points require notice, *viz.*—(*i*) general absence of the bacteria from the tissues ; and (*ii*) identity of the symptoms produced by the injection of only the products of the bacteria with those caused by the injection of the bacteria themselves. Now Cholera, which has come to be regarded as an intoxication process, is one of those diseases which does not quite conform to the first of these rules, as will be shewn below when we come to discuss it ; and Enteric Fever, in which the symptoms are chiefly due to the absorption of toxins produced by the bacilli locally, is another—many regarding it as a Septicæmic process.

CHOLERA.

Œtiology.—This disease, which is endemic in certain parts of India, and occurs in greater or smaller epidemics in other parts of that country and elsewhere, has had various causes attributed to it, but until Koch's discovery of the "comma-bacillus" the main fact of its œtiology remained unknown. Since this comma-bacillus is usually only found within the cavity of the intestines, cholera is generally regarded as a true intoxication process—the effect produced being due to the poison that is absorbed. As, however, comma-bacilli penetrate also into the tissues of the intestines, some authorities consider that the commas themselves are the chief producers of the disease. The difficulty in finding out the fact of this bacillus being in causal relation to the disease lay in this, that many other non-pathogenic bacteria are present in choleraic stools, and pathogenic micro-organisms are also met with in normal fæces ; but Koch was helped in establishing his comma-bacillus as being the true micro-organism of the disease by its property of growing and multiplying so rapidly, that in a case of Cholera the contents of the intestine practically swarm with it. A better name for it would be "Cholera-Vibrio," for although single individual bacilli look somewhat like commas, they are not thick at one end.

Morphology. Slightly curved rods, 1·5μ long and ·5μ thick, being about half as long as the Tubercle bacillus, but slightly thicker; occurring singly or in pairs—two individuals being so opposed as to produce an S-shaped curve—or in groups. On artificial media spirals may be formed. Their motile power is distinctly vigorous and rotatory, being brought about by means of a flagellum attached to one end of the vibrio. No spores are formed. Ordinary aniline dyes may be used to stain it, but Gram's Method is of no avail.

Conditions of Growth. The cholera-bacillus can be cultivated on ordinary media, but for its isolation from adventitious bacilli a medium is necessary in which it grows faster than they do. This is found either in 1 per cent. Peptone Solution; or in gelatine made alkaline with the addition of 1 per cent. Sodium Chloride. On gelatine plate cultivations (which require a temperature of 20°C.) the colonies present a characteristic appearance, being seen first as tiny grey-white dots just under the upper layer of the medium; and these on reaching the surface rapidly spread, affording a granular appearance, and causing gradual liquefaction of the gelatine in annular zones, with the formation of tiny pits, at the bottom of which lie swarms of wriggling cholera-vibrios. These pits under the microscope might at first be mistaken for numerous small air-bubbles. In a puncture cultivation in gelatine an extremely characteristic growth is obtained, liquefaction first appearing and being greatest at the surface, where the comma-bacilli are in contact with the air, the track of the stab of the needle proceeding downwards into the medium in a corkscrew fashion, so that the whole appears like a funnel with an elongated twisted neck. After about eight weeks all the micro-organisms have died, and transplantation is no longer possible in gelatine; but vitality is prolonged for a far greater period in Agar-Agar, in which medium, however, the growth is not characteristic. On broth and peptone solution the vibrios multiply rapidly, with the formation of a delicate pellicle on the surface. Growth is possible also in milk but, owing to the rapid onset of acidity, is only short-lived. In sterilised water the comma-bacilli grow rapidly at first, and are capable of existing for a considerable period; but in unsterilised water they are soon killed by the presence of other micro-organisms. Liquefaction occurs in blood serum, but the growth is not peculiar in any way.

A temperature of 16°C. is inimical to the cultivation of comma-bacilli, but even freezing for a short while does not kill them, though temperatures much below this point are immediately fatal. Very slight resisting power is shewn to desiccation, and any temperature above 55°C. will soon destroy them. In the moist condition vitality may be prolonged for months, but is at once de-

stroyed by the action of Carbolic Acid, Perchloride of Mercury, and other disinfectants. Weak solutions of acids, such as ·2 per cent. Hydrochloric Acid, soon kill the vibrios, and the basic products of a toxic nature and reaction, which are naturally formed by their growth, are neutralised. Sunlight is also rapidly fatal.

Detection of Cholera-Vibrios in Drinking Water, Fæces, etc.—This can be quickly done by taking 200 c.c. of the fluid to be examined (less in the case of stools), and adding this amount to 10 c.c. of broth, allowing the mixture to stand in an incubator at the body-temperature for 10 to 24 hours, at the end of which time plate cultivations can be made from the growth on the surface of the liquid in the tube. When present in relatively large numbers in choleraic stools, plate cultivations can be made directly.

Koch's Canons: Inoculation Experiments.

Canons Nos. I. and II. can be readily satisfied, but No. III. presents some little difficulty. Thus, with the exception of man, no animal naturally susceptible to cholera has been found. During an epidemic of the disease man alone dies, but if his digestion be in good order he may either not suffer from cholera or it will not kill him, possibly because the acid gastric juice of his stomach is inimical to the life of the cholera-vibrio. Now, during a cholera epidemic animals are never seen to be affected with even symptoms resembling the disease, nor can they be made to artificially develop it by the administration by the mouth of choleraic stools, pure comma-bacilli, or foods contaminated by them. Hence, in order to render a normal guineapig susceptible to the infection of cholera by the alimentary canal, Koch administered 5 c.c. of Sodium Bicarbonate solution through an œsophageal catheter, having previously injected into the abdominal cavity 1 c.c. of Tr. Opii to each 300 grms. body-weight of the animal, to quiet the stomach and prevent peristalsis. After this, on cholera-vibrios being given to the guineapig by the mouth, typical signs of the disease were obtained. As soon as the animal recovers from the administration, it refuses food, becomes weak in the limbs, then paralysed, and dies in about 24 hours; and post mortem the intestine is found filled with watery liquid stools in which hosts of the vibrios are discoverable. None are found in the blood. Subcutaneous administration, however, in the guineapig results in either a fatal septicæmia being developed, or only a local reaction; this being dependent on the virulence of the culture injected. Intraperitoneal injection kills the guineapig rapidly, the *post-mortem* appearances being somewhat like those above-mentioned.

Proof of the Comma-bacillus being the cause of Cholera.—The validity of Koch's cholera-vibrio being the cause of the disease has been more than established, and all the different theories of subsoil water as being the direct and primary factor in causing it—(although they may hypothetically be regarded as predisposing to

an outbreak)—have been absolutely overthrown. If more proof were needed, it was only too forcibly afforded in the recent Hamburg epidemic of 1892-1893. In the great summer epidemic, out of 18,000 people in Hamburg who drank impure unfiltered Elbe water more than 8,000 died. Those living in Altona, only slightly lower down the stream, also drank Elbe water; but as it was carefully filtered first, they escaped free of the disease. In the winter epidemic, however, Altona suffered as well as Hamburg, and the cause of this was found to be due to a leak in the filter-beds of the Altona water-supply—brought about by the great frost—which were therefore not acting properly. Koch proved this, and also shewed that the epidemic of cholera which occurred in winter, in the Niebleben Asylum, which is situated on a hill of porphyry, was due to the inmates drinking cholera-bacilli in sewage-farm filth, which had been allowed to enter a stream at a point higher up than that from which their water-supply was drawn—the intense cold preventing proper filtration both in the sewage farm and in the filters of the asylum.

Chemical test of a growth of Cholera-Vibrios.—If 1 c.c. of Sulphuric Acid be added to a pure cholera cultivation in Peptone, a red coloration is obtained, due to the liberation of Nitrous Acid from the nitrites formed by the action of the vibrios on the albuminous contents of the tube. Sphinckler's Vibrio (which resembles Koch's Comma-bacillus in shape) will not give this test, nor can it be obtained from Enteric Fever or Anthrax bacilli; but the Vibrio Metchnikoff and certain other bacteria will afford the same result, so that it is not altogether a diagnostic test of a cholera-growth. The pure vibrios and pure sulphuric acid must be used, for otherwise the nitrites may be the result of contaminating bacteria or exist as an impurity in the acid. Indol is detected by its smell in cholera-cultures.

Immunity in Cholera.

Natural immunity does seem to occur, some persons being apparently more or less immune; but this may possibly be due to variations in the virulence of the vibrios taken in, some being extremely powerful, others practically powerless in the production of the disease.

Artificial immunity may be produced—(*1*) *By the injection of an antitoxin.* Kitasato has recently discovered an anti-cholera serum which is antitoxic, in the strict sense of the word, in guineapigs—·2 c.c. of the serum being sufficient to neutralise the effect of ·2 c.c. of sterilised broth culture of cholera, when injected simultaneously into the peritoneal cavity. Pfeiffer found that by injecting cholera-vibrios into a goat he could obtain an antitoxic serum, which, however, was of little avail against intestinal cholera. The same observer has also shewn that the blood of an animal immunised by repeated injections of cholera-vibrios pos-

sesses a germicide action quite distinct from the action of antitoxin, as it kills the germs themselves, whereas the antitoxin counteracts the poison which the germs form. Hence such serum can be used as a test (*cp.* Enteric Fever); and if on its addition to a growth the micro-organisms are killed, these micro-organisms are (in all probability) cholera-vibrios. In the Pasteur Institute recently, a different antitoxic serum, obtained from an animal to whom the toxines alone, derived from a recent cholera-culture, had been given, is said to have neutralised the effect of intestinal cholera in sucking rabbits. The separation of the toxines from a cholera-growth is performed by intraperitoneal osmosis from cultures enclosed in animal membranes and is a matter of such extreme delicacy that more knowledge is needed on the subject before a decided opinion can be given. Kitasato lately used his serum on cases that occurred at Tokio in Japan with very beneficial results. (*II*) By inoculating dead cultures of cholera bacilli into animals they were found by Pfeiffer to be rendered immune against Cholera. It has been suggested that this method, being less dangerous, should be used instead of the following in Anti-cholera Vaccinations in Man.

(*III*) *Haffkine's method of Cholera Vaccination*, the principle of which may be considered to be to establish a sufficient tolerance of the cholera virus to allow of cure taking place, on the onset of the disease, by expulsion of the vibrios from the intestine—*i.e.* to make the animal poison-proof and thus indirectly bacteria-proof. As, however, the poison in Cholera seems to be so intimately connected with the growth of the comma-bacilli and so difficult to separate from them, the process is simultaneous and is brought about, not by the injection of the toxins, but by the injection of cultures of pure cholera-vibrios. The method first consisted in the administration by hypodermic injection of a first vaccine of attenuated cholera-vibrios (produced by growing in conditions of intense oxidation and at too high a temperature); and following this up by injection of a second vaccine of cholera-vibrios of maximal virulence.

The culture of attenuated cholera virus is obtained by growing cholera-bacilli at 39°C. in the presence of constantly renewed oxygen, an attenuated culture being thus realised which may be propagated by growth in ordinary conditions, always remaining attenuated.

The exalted cholera virus, is obtained by a series of passages of the vibrios through the peritoneal cavity of guineapigs, with interoculated intervals of aerobic growth—taking great care, by antiseptic precautions, in inoculating the animals, and frequently examining by stroke-cultivations on Agar-Agar. By this means a strength equal to 32 times that of the original tube-growth may be eventually reached; though this depends on the original strength of the virus.

Two varieties of vaccines are prepared from pure cultures of the cholera-vibrios on Agar-Agar—(*i*). Living vaccines, emulsified in sterilised broth or water; and (*ii*). Carbolised vaccines, containing ·5 per cent. Carbolic Acid (which has been previously sterilised: for although this amount of the antiseptic is quite sufficient to kill off the Cholera-vibrios, it does not destroy other adventitious bacilli which may be living in such a weak solution of the antiseptic itself). Professor Haffkine has, however, lately abstained from using Carbolised Vaccines in practice, as likely to give less lasting results than Living Vaccines; and he has also modified his process with regard to these latter, as will be shewn later on.

The Symptoms produced by Inoculation.—A dull pain comes on in 1½ to 3 hours after the injection and is accompanied by swelling and redness of the area around. In 5 to 7 hours or so, fever has supervened, being occasionally ushered in by one or more well-marked rigors, nausea, and headache. The pain now becomes very severe and continues so, with increased local swelling, for about 2 days; but the temperature, which rarely rises above 102°·5 F., subsides to normal or thereabouts in 20 to 36 hours, though the headache and malaise persist for some while longer. After the pain subsides a small indurated swelling remains under the point of inoculation, which disappears in the course of a few days. Sometimes the febrile symptoms are more severe, and painful swelling of the lymphatic glands adjacent to the point of inoculation takes place, but suppuration never results.

Owing to the local symptoms and fever caused by the inoculation, many people, after having received the first vaccine, did not present themselves for the second. Haffkine accordingly has modified his process and now omits the first vaccine, giving a smaller dose of the second instead, and following this up in some few cases, with a further inoculation of the second vaccine.

The method employed is as follows:—(1) Having grown the most virulent Cholera on Agar-Agar, protected from direct sunlight, for a time not exceeding 24 hours, and each tube having been examined microscopically to ascertain that the growth of the vibrios is perfectly pure, an emulsion is prepared by aseptically introducing sterile water (or broth) into the cholera-culture tube, under the strictest precautions. The amount of water introduced is roughly one-third up the height of the slanting Agar-Agar, and is gently shaken and moved over the surface of the medium till all the vibrios have been washed off—a milky liquid resulting—and the surface of the medium left perfectly clean. (2) This milky liquid is carefully sucked up into a previously sterilised hypodermic syringe graduated off into scales. (3) Hypodermic inoculation into the subcutaneous tissue of the left loin is then performed under strictly antiseptic precautions. The dose of the emulsion injected is ½ c.c. (♏ ix) for an adult; ¼ c.c. (♏ iv—v) for a child of 10 years; and $\frac{1}{20}$ c.c. (♏ i) for a baby of 6 months.

For preparing the Carbolised vaccines ·5 per cent. watery solution of Carbolic Acid is used to make the emulsion, which is then increased by adding up to 8 c.c. and drawn up into 8 sterilised vaccine tubes, each capable of holding 1 c.c., and sealed off; in which way they are kept until required for use, when the outside of the vaccine tube having been first sterilised by heat and the emulsion within well shaken up, the end of the tube is broken off and everted over a sterilised syringe capable of holding 1 c.c. until the latter is full, and the inoculation performed as described above. The carbolised cultures can be kept indefinitely, and so can be sent long distances. Over 65,000 people have now been inoculated, mostly by Haffkine himself, against Cholera, and the results are said to have been uniformly successful and encouraging. Even when small doses of a weak vaccine were employed, the number of deaths was stated to have been reduced to one half (Gaya Jail); while amongst the Assam coolies, on whom strong doses were used, the death-rate was returned as reduced to one-sixth. After a small dose of weak vaccine protection was found, in Lucknow, to have resulted 15 months after the inoculation was made; so that, in all probability, when a strong dose of the most powerful culture is employed, protection ought to be afforded to the inoculated for several years. In Calcutta lately it was found that after moderate doses (such as the method described) the proportion of deaths amongst the inoculated and uninoculated who afterwards suffered from cholera was as 1 to over 20; and such a result is indeed marvellous when one considers that by ordinary medicinal treatment in an outbreak of Cholera in India a mortality of anything under 40 to 50 per cent. of cases attacked is regarded as distinctly favourable.

Transmission of Cholera.—It is a water-borne disease essentially, but is also capable of being transmitted by means of milk (*see* above); on fruit, vegetables, and other articles which have been contaminated by being washed with infected water; and through the agency of flies, rats, and other animals, as well as on damp clothes and suchlike materials. A direct case of infection is reported from Koch's laboratory in the person of an investigator who was experimenting with cholera-vibrios; and another from the bacteriological laboratory in Hamburg.

Precipitation Tests in Cholera.— Pfeiffer and others have found that cholera bacilli, when mixed with the serum of an animal rendered immune against the disease, are precipitated and agglutinated. (For methods, see under the heading of Enteric Fever.)

Blachstein has also noticed that Chrysoidin precipitates cholera bacilli when they are in suspension. No other substance except the serum of those immune against Cholera is known to do this, and as Chrysoidin is a non-poisonous substance, it can be made use of as a bactericide practically against Cholera-bacilli.

ENTERIC FEVER.

The bacillus of this disease was discovered by Eberth in 1880, and has been found in the Spleen, Peyer's patches, and mesenteric glands. Though it has been seen in the blood taken from the subcutaneous rose spots, it is not met with in the lumen of the intestines until after the 8th or 9th day of the disease, and often not until marked ulceration has occurred ; but it has been isolated from the urine of patients suffering from Typhoid Fever.

Morphology. A short bacillus, with rounded ends, usually thick, 2μ to 4μ long and ·5μ to ·8μ broad ; extremely motile, especially in neutral bouillon cultures, being provided with lateral cilia and distal small flagella, ten to twelve in number. To stain the bacillus in sections Carbol-fuchsin answers better than other aniline dyes ; but for ordinary purposes Loeffler's Blue will clearly define both the cilia and flagella. Gram's Method is not available. Under all conditions the bacillus parts with its stain very readily, though Tannic Acid will greatly help to fix it.

Conditions of Growth. In the tissues it is seen singly or in pairs, but artificially cultivated it may grow out into threads. The powers of resistance of this bacillus are extremely great. Cold, or even repeated freezing and thawing, does not appear to affect it ; heat, however, is more powerful, a temperature about 65°C. being fatal. In distilled water and in the upper layers of the soil the bacillus can exist for some months, but in ordinary water it is outgrown by other more vigorous saprophytes. Even ·1–·2 per cent. Carbolic does not produce any visible effect on its growth, and this fact of resistance to Antiseptics is made use of in obtaining pure cultures of the bacillus. Thus ·05 per cent. of Carbolic Acid added to Gelatine forms a medium, which when melted, is inoculated with the faintest trace of Enteric fæces (2nd and 3rd week) or spleen-pulp of an animal dead of the disease. One drop of this mixture, after proper stirring, is transferred to another tube : from which again one drop is transplanted in a third tube. The contents of each tube are poured into a Petrier's dish, and plate cultivations thus obtained of the Typhoid Bacillus and also somewhat of the Bacillus Coli Communis ; but all other micro-organisms are destroyed by the Carbolic Acid present. The colonies are sharply circumscribed, sometimes round, sometimes wedge-shaped, yellow-brown in the centre, and do not cause liquefaction of the gelatine. At first it may be difficult to distinguish between the colonies of the Typhoid bacillus and Bacillus Coli Communis, so further transplantation may be necessary for this purpose. In a gelatine stab-culture colonies form along the track of the needle and run together, without liquefaction of the medium occurring ; and an irregular small whitish layer forms on the surface but

does not spread much. On potato a characteristic glaze-like transparent glutinous layer is formed by the end of the second day, and is made up of Typhoid bacilli. (This only occurs if the reaction of the potato is acid ; if it be neutral or alkaline a yellow-brown layer will be produced instead, resembling the growth on potato of Bacillus Coli Communis.) In milk acidity does not seem to interfere with growth, but no coagulation is observed.

Infectivity in Enteric Fever has been settled by epidemic occurrence of the disease, which is caused by the toxine produced by the bacillus in the tissues. As this micro-organism is not only parasitic but saprophytic as well, its propagation outside the human body must be carried on in various media. Chief amongst these is milk; but dust, air, oysters, vegetables moistened with infected water, water itself, sewage matter, soiled clothes, etc., also serve ; whence the direct or indirect conveyance to man and subsequent ingestion by him occurs.

The mode of infection is practically invariably by the digestive tract, and the resisting power of the bacillus may enable it to pass through the acid contents of the stomach into the intestines, where the chief local lesions occur. The bacilli enter Peyer's patches and the solitary glands of the large intestine, in which they multiply during the incubation period of the disease, developing their toxines and causing local leucocytosis, with swelling and eventually necrosis and sloughing of these parts. Thence they pass by the lymph-channels to the mesenteric glands, spleen, liver, and perhaps kidneys, causing enlargement of these organs and albuminuria by their presence in the last-named. They always occur scattered about in small masses ; and may occasionally enter the blood-stream, though under ordinary circumstances this does not happen, unless their presence in the rose-pink spots that often appear on the skin in the earlier stages of the disease is accounted for in this way.

Application of Koch's Canons.—I. As the bacillus is not always readily found the first canon is somewhat difficult to satisfy. II. The bacillus is easily grown outside the body. III. Although the guineapig is the most susceptible animal, contradictory results have been obtained from the result of inoculation of Typhoid spleen-pulp into its peritoneal cavity. (See later on.)

Difficulty of classification of the disease, from a bacteriological point of view, occurs from the contradictory results obtained experimentally in animals. Some regard it as an intoxication process, due to poisons being produced by the bacilli in the cavity of the intestines and subsequently absorbed into the system ; but this view of the position where the toxines are formed is manifestly incorrect, as the bacilli are not met with free in the intestines when the first symptoms of the disease appear. Since, however, the bacilli are intra-systemic, being demonstrable in Peyer's

patches, the spleen, etc., they most probably generate their toxines in these localities, which on absorption produce an intoxication and give rise to the intestinal symptoms of purging, etc., in man ; and indeed the same symptoms and intestinal lesions can be obtained in the guineapig by injection of the toxin, so that some consider it justifiable to look upon Enteric Fever as an intoxication process. Others there are who consider it to be a Septicæmia, since in rats, guineapigs, etc., injection of the very virulent bacilli is followed post mortem by their appearance in the blood ; while the phenomena of necrosis and sloughing seen in the intestines might lead to the disease being regarded as a local destructive process. Latest views on the subject tend to shew this last theory to be the correct one. A local inoculation occurs by the intestine, and may remain local or the bacilli may spread to the mesenteric glands, causing their enlargement, and from either situation emboli may reach the spleen, skin, other glands, lungs, or other parts of the body. In the spleen the bacilli are found in embolic masses, not disseminated throughout the organ (which one would expect to be the case if phagocytosis had occurred in the blood); and the disease itself is never really a Septicæmia, as is proved not only by the above fact, but also by the fact that while the bacilli can be found in a drop of blood squeezed out of a "rose-spot," yet they are not found in the blood simultaneously taken from any spot near by or from the finger—*i.e.*, the rose-spots are really minute subcutaneous emboli in their origin. The disease can therefore be regarded as a local process, the bacilli being liable to dissemination as emboli, not freely through the blood, and the symptoms, intestinal or other, being produced by the absorption into the system of poisons locally produced (Intoxication Process). These differences in the explanation of the disease are probably due to a varying degree of virulence of the bacillus ; so, in order to obtain, as far as possible, constant results in experiments, there is need for exalting the disease.

Inoculation Experiments.

Mice, rabbits, and guineapigs are susceptible as well as man ; but the vast majority of animals exhibit a marked resistance to the disease. If the contents of the stomach are made alkaline, and a large quantity of laudanum injected intra-peritoneally (as in Cholera), some observers found that on introducing Typhoid bacilli through a catheter into the digestive tract of animals, results, much like the disease in man, are obtained ; and especially if the virulence of the bacillus is increased.

Method of exalting the power of the bacillus.—(*i*) By diminishing the resistance of the organism to the micro-organism, as by the simultaneous injection of saprophytic bacteria—(Bacillus Prodigiosus) ; and (*ii*) by increasing the virulence of the micro-organism by the "Method of Passages"—*i.e.*, by injecting the peritoneal fluid,

which swarms with the bacilli, of one animal (guineapig especially favourable) dead of the disease into the peritoneal cavity of another animal ; and so on.

Clinical Symptoms in Guineapigs.—After a virulent culture has been injected into a guineapig there is a rise of temperature for a few hours which is succeeded by a fall, and the temperature remains depressed until death occurs. Swelling and tenderness of the abdomen and malaise are the chief symptoms. Post mortem, exudation fluid of a sero-purulent nature, containing fine shreds of fibrin, is observed in the peritoneal and pleural cavities, and the intestines, Peyer's patches and abdominal viscera are swollen and congested ; the bacillus being found in the spleen, liver, adrenals, serous exudations, and sometimes also in the blood. In some cases, however, injection only produces a local result—the formation of an abscess at the spot of inoculation, and the animal recovers.

Question of Immunity.

Animals have lately been " accustomed " to the Typhoid bacillus, and by this process an anti-toxic substance obtained from their blood, which can be used on other animals for protective purposes. In a few cases also it has been reported that the blood of persons who had nearly recovered from Enteric Fever contained a substance which had a markedly protective effect on guineapigs. The subject requires further investigation, but there seems to be no doubt that an antitoxic material will shortly be forthcoming which will be used in the general treatment of the disease in man ; and indeed inoculations by means of a vaccine containing dead Typhoid bacilli are now being carried out in Europe against Enteric Fever ; while Kolle and Pfeiffer have obtained a similar vaccine, prepared from a culture taken from a typhoid spleen, which on injection into animals, is said to have rendered their serum protective.

Serum Therapy.—Chantemesse and Widal found that the serum of animals inoculated with Typhoid bacilli in dead cultures was preventitive, but not curative. In order to obtain sufficient antitoxic power, he grew extremely active pure Typhoid bacilli on culture media of spleen, bone marrow, and a little human blood. These produce a virulent toxin, which is at its maximum on the fifth day of growth ; and in a rabbit the dried residue of culture produces great diarrhœa when hypodermically inoculated. Progressive doses of this toxin (the bacilli being killed off) are injected into horses, and at length 60 c.c. can be injected without much reaction. At this time the horse's serum is found to be markedly antitoxic, not only as seen in animals, but also by treatment of the disease in man. Further experiments in mankind are, however, necessary before a definite and conclusive opinion can be formed as to its value,

Diagnostic Tests of Enteric Fever.—(See also Chapter XI.) (*i*) *Pfeiffer's test.*—Serum of blood taken from a typhoid patient is added to a growth of Eberth's bacillus, and the mixture injected into the peritoneal cavity of a guineapig. The bacilli are rapidly immobilised, undergo granular degeneration and are dissolved. (*ii*) *Wyatt Johnston's Test.*—If a drop of dried typhoid blood be dissolved in a drop of distilled water and the mixture added to a drop of actively motile typhoid culture—preferably not over 24 hours old—the motion rapidly stops, and the bacilli, in from a few minutes to 24 hours (longer the less concentrated the sample) run together into loose coils or clumps. This result can also be obtained with a drop of dried typhoid serum or blood, even after having remained dry for 60 days, and is typical in contrast to the result yielded when a drop of normal serum is added to a drop of similarly active typhoid culture, in which case the motion, after a brusque stoppage, re-appears with increased activity. The reaction is sometimes seen as early as the second day of the disease, always on the 5th or 6th, and is most marked after the first week. This is distinctly more convenient than (*iii*) *Widal's Test*, where the liquid serum obtained from the blood of a typhoid patient is added to and mixed with virulent typhoid bacilli, either in a hanging drop under the microscope (Pfeiffer, Bordet), or in a small tube, *e.g.*, a narrow test-tube. Precipitation, agglutination, and agglomeration of the bacilli are seen, and other changes as described. The liquid above becomes clear.

Intestinal Antiseptics in Enteric Fever.—Since the typhoid bacillus lives within the system, local disinfectants applied to the intestine cannot reach it, although they may affect other bacilli. It was found experimentally that the number of typhoid bacilli in a measured quantity of stools did not diminish after the clinical administration of disinfectants; nor were intestinal antiseptics effectual in killing off the Bacillus Prodigiosus after its administration to animals. It would therefore appear that local intestinal antiseptics are of little use, if of any, in Enteric Fever, since the disease is due to the action of the toxins produced. Attention should, however, be paid to the urine.

BACILLUS COLI COMMUNIS.

This bacillus, which was discovered by Emmerich, is frequently found in man in peritonitis, ischio-rectal abscess, etc., and although it is to be also met with in the intestinal canal it is non-pathogenic in that situation, being in fact always present in human fæces and also in the dejecta of some of the lower animals.

Morphology. Short rods, much resembling the Typhoid Bacillus, 2μ long and ·5μ broad, occurring singly or in pairs, and staining with ordinary aniline dyes, but not by Gram's method.

Conditions of Growth. In a gelatine subculture a leaf-like flattened colony is found on the surface, with beads along the track of the needle. The best temperature is 37°C. Both aerobic and anaerobic growths can be obtained.

Distinction from the Typhoid Bacillus.—The Bacillus Coli Communis is extremely like the Typhoid Bacillus and frequently contaminates its cultures, but the following points serve to distinguish between them—(*i*) On acid potato B. Coli Communis produces a brown growth like that of the Typhoid Bacillus on alkaline potato; (*ii*) when grown in milk coagulation is promptly caused—this does not occur with the Typhoid Bacillus—(*iii*) when grown anaerobically in Gelatine or Agar-Agar containing a small quantity of glucose, gases are produced; (*iv*) Indol is produced by B. Coli Communis, but never by the Typhoid Bacillus; and (*v*) when grown on 25 per cent. gelatine B. Coli Communis produces turbidity much more slowly than does the Typhoid Bacillus.

Inoculation Experiments. When injected into the circulation of a guineapig, B. Coli Communis causes death in 24 hours. When injected under the skin or into the peritoneal cavity, a small dose produces only a local effect, but a large dose causes fever, and diarrhœa, followed by collapse and death.

MALTA FEVER.

Œtiology.—A disease peculiar to Malta and certain shores of the Mediterranean, whose micro-organism was discovered by Surg.-Capt. D. Bruce, A.M.S. Clinically it much resembles Enteric Fever, but is distinguished by the absence of rose-spots on the abdomen and lesions in the intestines, by the longer duration of the disease, and by the tendency to inflammation of the joints and testicles. In many respects it resembles Malarial Fevers also. The micro-organism causing it is a micrococcus—the Micrococcus Melitensis.

Morphology. A micrococcus, round or slightly oval in form, and ·33μ in diameter; possessing active molecular movement, but no spontaneous powers of motion; occurring singly or in pairs, never in chains. It can easily be stained by ordinary basic aniline dyes, but Gram's Method is not applicable.

Conditions of Growth. The best culture-medium is 1·5 per cent. peptonised Agar-Agar beef-jelly. If a stab-culture be made from the spleen-pulp of a fatal case and kept at 25°C., after seven days minute white

droplets appear around the puncture, which in the course of weeks enlarge into a rosette-like growth, while the needle-track appears as a yellowish-brown solid-looking uneven growth in the medium. No liquefaction occurs in a gelatine culture.

Inoculation Experiments. Besides man, the monkey is the only susceptible animal that has yet been found. Subcutaneous inoculation of the micrococcus of Malta Fever in a monkey rapidly causes high fever followed by death in 13 to 20 days, and post mortem is discoverable in the spleen, as in the case of man.

Koch's Canons.—I.—The micrococcus is found in the spleen in all cases of the disease, and is not known under any other condition. *II.*—It can be grown outside the body. *III.*—Susceptible animals are found in "Bonnet" monkeys which can readily be inoculated with the disease.

DIPHTHERIA.

The infectivity of Diphtheria has been proved beyond all doubt to be due to the bacillus discovered by Klebs in 1883, and isolated and cultivated by Loeffler in the following year. This bacillus is always present in the throats of patients suffering from the disease, and may be coughed or sneezed into an uninfected throat or nasopharynx. It is also to be found in the throats of patients for some weeks after recovery has begun, and of those who have attended on them. This latter point, as well as the former, accentuates the need of immediate effectual and prolonged isolation in cases of the disease.

The clinical symptoms are characterised by the formation of "false membranes" in the nasopharynx, larynx, and elsewhere (in Germany the intestines are not unfrequently implicated, though this lesion is seldom if ever seen in England); great exhaustion, swollen glands, and subsequent paralyses.

Seat of the Bacilli.—As a rule, the bacilli do not leave the region of the deeper layer of the mucous membrane, but some fatal cases may possibly be complicated by a more or less general infection. Pneumonic infection, which may occur, certainly supports although it does not prove this view. Other adventitious bacilli, found on the surface of the diphtheritic membrane, are not the true cause of the disease.

Morphology. In form the Diphtheria Bacillus much resembles the Tubercle Bacillus, being about the same length, but nearly twice as broad. It is, however, very irregular in shape, varying in length, being either straight or slightly curved, beaded or sometimes club-shaped, and having its ends either pointed or rounded. In cultures great irregularity of form

and size is observed. It is non-motile and does not possess flagella. Spore-formation does not occur. Gentian violet, or Loeffler's Blue, if heated a little, readily serves to stain it, but Gram's method only acts with difficulty.

To obtain the bacilli for inspection, a tiny bit of the false membrane is removed from the fauces by means of cotton wool on the end of a probe, dried on a filter-paper, and teased finely on a coverslip ; then fixed, stained, and examined.

Conditions of Growth.

Glycerine Agar-Agar, sterilised serum mixed with broth and 1 per cent. glycerine, and boiled white of egg, are the best media for its separation from contaminating bacilli ; stroke cultivations being made, and the colonies of diphtheria bacilli appearing in 24 hours as small round greyish-yellow discs before the other bacilli have had time to develop. Its cultivation may afterwards be continued in ordinary media, *e.g.*, in broth where, after causing a transient cloudiness, it grows in small flocculi at the bottom of the tube. If no glucose be present in the broth, more luxuriant growth of the bacilli and greater production of toxin occurs than if there is. Milk is a very excellent medium for cultivation of the bacilli, and if litmus be first added the change from alkalinity to acidity and back again to alkalinity as the culture grows old can be plainly watched. It can also be grown on potato as a thin glaze. The Diphtheria Bacillus is a facultative anaerobe ; and if grown in presence of a current of air its luxuriance is much increased although the duration of its existence is shortened ; but it can also be grown out of air. By its great resistance to drying another means is afforded for its isolation from contaminating micro-organisms.

To obtain a growth from the false membranes, either stroke-cultures may be made at once, or a small bit of the membrane may be dried at 37°C., by which means most adventitious bacteria are killed off, and stroke-cultivations made in at least three serum-mixture tubes with a little taken off on a needle. After being kept for 12 hours at 37°C., the irregularly outlined yellowish-grey-white colonies may be examined.

Koch's Canons.—I.—The first canon is not easily satisfied inasmuch as—(*a*) there is difficulty in recognising the particular bacillus amongst a number of saprophytic bacteria, and (*b*) in distinguishing the particular pathogenic bacillus from similar non-pathogenic ones. *II.*—Very favourable culture-media are required to isolate the specific bacillus. However, the Diphtheria Bacillus will outgrow all other bacilli on sterilised serum or white of egg, and can thus be fairly easily isolated if stroke-cultures are made. *III.*—Some difficulty is experienced in reproducing all the symptoms of the human disease in animals, for

they often die after inoculation with Diphtheria without manifesting paralyses. Hence, Loeffler himself formerly had some doubts as to the real specificity of the bacillus.

Roux and Yersin found the bacillus in 61 out of 80 supposed cases of diphtheria. Of these 61 cases 30 died. The 19 that remained out of the 80 recovered. Hence, they concluded that only such false membranes as those in which the Diphtheria Bacillus can be found are to be regarded as true instances of Diphtheria.

A similar bacillus—*Pseudo-Diphtheria Bacillus*—was found by them in number of healthy mouths, especially of school children. This bacillus is morphologically almost exactly like the true bacillus of diphtheria, but is not fatal to a guineapig on inoculation. Roux thought at first that Pseudo-Diphtheritic bacilli were only true Diphtheria bacilli which had lost their virulence; and so he made an attenuated form of the true bacilli by passing air through the medium in which they were growing; but after reaching a certain stage of attenuation, he found that the virulent form could not be regained. Nor was he able to make the false bacilli virulent: so there probably is not the slightest connection between the two. Working still further, Roux discovered that if the dose of the true bacilli given to an animal be not too strong, then paralyses will follow.

Inoculation Experiments. Susceptible animals are found in guineapigs, rabbits, dogs, cattle, sheep, and cats. The horse is somewhat tolerant of the bacillus. If ·5 c.c. of a broth culture of 24 hours' growth be hypodermically inoculated into a guineapig extensive local œdema results; and after death, which ensues in a day to a day and a half, the liver is found enlarged and contains small necrotic areas, while the lymphatic glands, adrenals and kidneys are also swollen, and fluid is discovered in the pleural cavities. Sometimes the bacilli are seen in the blood, but very rarely. If a rabbit's trachea be inoculated after tracheotomy has been performed, a typical false membrane results and paralyses follow; and the same end can be obtained by inoculation on the rabbit's ear after the epidermis has been scraped away.

If instead of using the pure bacilli in the guineapig the toxin be substituted, identical results occur.

Virulence.—Diminution of virulence is obtained, as in cholera, by aeration of cultures at 37° to 39°C.; and the virulence is tested (*a*) by rate of growth in the most favourable media, and (*b*) by the effect of the poison, in rate of time, in causing the death of a guineapig.

Nature of the poison produced.—Though usually regarded as an albuminous substance, the toxin of Diphtheria can be developed in non-albuminous solutions or urine. Its greatest virulence is seen in cultures of the second and third week.

Immunity. Antitoxines of Diphtheria.

Behring found that animals could be rendered immune against the disease by inoculation first with attenuated cultures and then with more virulent ones of the bacillus, and that their blood contained an antitoxin, capable of being extracted from their serum, which afforded protection against the disease on inoculation into unprotected animals.

Preparation of the Toxin.—Virulent diphtheria bacilli are grown in alkaline bouillon for 3 or 4 weeks, at 37°C., with a stream of moist air constantly passing over the cultures. First of all an acidity is produced, after which a far greater alkalinity than originally existed results. Tricresol is then added to the extent of ·4 per cent. to the cultures, which are afterwards filtered through porcelain, the toxin being contained in the filtrate. This should be of such a strength that ·1 c.c. will be fatal to a guineapig weighing 500 grms. in 24 hours, but it is very difficult to procure, and the average toxin kills in 30 hours at the same dosage.

Immunisation of the animal.—Only those animals which naturally present a distinct immunity to ordinary doses of the toxin, and are big enough to furnish a fairly large quantity of the antitoxic serum should be used. Accordingly, Behring chose sheep and dogs ; but horses are now generally used for this purpose instead. Any maimed horse, provided it be free from rheumatism, glanders, and tuberculosis, will do (to prove this it is injected first with Mallein and afterwards with Tuberculin), but recent investigations show that a well-bred horse in good condition will stand the inoculations better and yield a far greater supply of antitoxic serum, which is also more efficacious than that obtained from a coarse low-conditioned animal. A first small and carefully measured dose of the toxin is injected, and, if well borne, is followed by the injection of a larger dose after the lapse of eight days, and after another eight days by a still larger one, until quite enormous doses are injected at one time. The injection causes local œdema and febrile reaction ; but if made fairly slowly, these symptoms are distinctly less marked than if performed rapidly. Care is required in watching the animal, for, if too frequent or too rapidly given, the doses will produce cachexia and death. By mixing Trichloride of Iodine or Gram's fluid with the toxin its irritant effect is greatly reduced. At various stages of the immunisation the antitoxic power possessed by the serum of the animal is tested, and the process carried steadily on until the serum possesses an antitoxic power of 500 to 700 units per 10 c.c. (see below), a result which is obtained in 6 to 12 months by Behring's method, but possible of being attained in 4 to 6 months if a more powerful toxin be employed for the purposes of injection, as in Roux's method.

Preparation of the Antitoxic Serum : Graduation of its Strength.—As soon as very large doses—250 c.c. or so—are capable of being injected into the horse without very marked effect, the ani-

mal's blood is in an antitoxic condition. It is accordingly bled from the jugular vein, and the blood allowed to flow through a cannula into aseptic sterile bottles which are placed on ice, and on being left for a day or two like this, the clear *antitoxic serum* separates from the coagulum and can be pipetted off. As the strength of this serum varies, it is expressed in terms of *immunising units* as compared with *Behring's normal serum*, which is of such a strength that 1 c.c. simultaneously injected with 10 times that amount of toxin into a guineapig, will protect the animal. One c.c. of such a serum is regarded as one immunising unit, and is therefore equal to ten antitoxic units. Ordinary commercial serums are of three strengths—(*a*) 600 units in 10 c.c. (used in people who are not suffering from, but come in contact with the disease) ; (*b*) 1,000 units in 10 c.c. ; and (*c*) 1,500 units in 10 c.c. (used in treatment of the disease). It is, of course, not possible to form an accurate dose, since no knowledge of the amount of toxin present in the patient and requiring to be counteracted can be afforded ; but a full rather than an under-dose should be given, since the serum produces no fatality. Statistics show marvellous results of this treatment, and the death-rate in Diphtheria has been reduced 30 and 50 per cent. by its adoption ; but it must be resorted to early, for once destructive lesions have begun in the organs and tissues, it cannot reasonably be expected to succeed, and must of necessity fail. The action of the serum may be either—(*a*) Preventitive, by encouraging phagocytosis, or (*b*) Antitoxic, by neutralising the poison ; but probably the former is the chief factor in the clearing away of the membrane—as seen, say, in a child—and indeed, such phagocytosis was found by experiment to occur in the anterior chamber of a rabbit's eye which had been injected with the Klebs-Loeffler Bacillus after the animal had first been given a dose of antitoxin—the bacilli being shut in by leucocytes—while in a control animal, to which no antitoxin had been administered, the bacilli were found free.

Klein's Method of Preparing Diphtheria Antitoxin.—First of all unfiltered attenuated Diphtheria cultures are injected, and later on, large quantities of more and more virulent bacilli in successive doses. By this means the bacilli can grow and multiply, and a powerful antitoxic serum is said to be able to be obtained in 23 days.

Preparation of Antitoxin by Electrolysis.—Diphtheria antitoxin can be prepared from Diphtheria toxin to which ·5 per cent. Sodium Chloride has been added, by electrolysis—Carbon electrodes being employed. After chlorination has proceeded for some time, the Chlorine is removed by means of a repeatedly changed silver electrode which is put in to replace the Carbon electrode of one pole. A standard Caustic Potash solution is afterwards added, and a weak antitoxin is thus obtained, about $\frac{1}{50}$ the strength of that used by Roux.

Effect of Injection of Antitoxic Serum in a case of Diphtheria.—Practically, no effect is seen for 24 hours ; but after that the faucial swelling and nasal discharge lessen and subside ; the membrane becomes thinner, and is loosened, disappearing altogether in 4 or 5 days, while its extent is at once limited. The symptoms of fever, etc., disappear *pari passu*. Sometimes, though rarely, the serum produces high temperature, eruptions on the skin, or pains in the joints, after its injection. It is not proved that paralyses occur more frequently after its use than without it ; and certainly this does not appear to be the rule, though some observers appear to have noted it. Dried serums are far inferior in their efficacy and promptness of action to fresh ones.

TETANUS.

Œtiology.—The cause of this disease is a bacillus which was discovered by Nicolaier in 1884, and first obtained in pure culture by Kitasato in 1889. It probably invariably gains an entrance into the body (where it exerts its evil effect by the poison it produces, and not by its mere presence), by means of a wound or skin abrasion, though one case is on record of the bacillus being present in the intestine of a patient who died of Tetanus.

Morphology. This bacillus presents marked pleomorphism, due to different stages of growth, three forms being recognised—(*i*) Straight rod—which may occasionally grow into leptothrix ; (*ii*) Club-shaped bacillus ; and (*iii*) Drumstick-shaped bacillus (practically the only form which is seen in cases of the disease itself), which is spore-bearing in the head of the drumstick. This diversity of form accounts for the bacillus not being recognised at first. It is slightly motile in the first stage, immotile in the second and third. This has been quite recently shown by Kanthack to be due to the fact that in new cultures the bacilli are provided with flagella, lateral and terminal, some thick and others thin, 20 to 30 on each bacillus. In old cultures only one terminal flagellum can be made out. The movement observable in a "hanging drop" is sluggish, probably because the examination is not carried out under anaerobic conditions. The flagella appear to be prolongations of the protoplasm of the bacillus, passing through its capsule in minute channels which can be defined by staining with silver nitrate solution. They all disappear before sporulation takes place. The bacillus stains readily with ordinary basic aniline dyes or by Gram's Method. The spore takes much longer to stain and retains its colour much better than the rest of the bacillus. Hence, double-staining can be used, first by Carbol-fuchsin which stains the whole and is removed from all but the spore by washing in dilute acid, and then by Methylene Blue which colours the rest of the bacillus.

Conditions of Growth.

The tetanus bacillus is difficult of cultivation, though it can be grown on ordinary media. Gelatine to which 2 per cent. of grape sugar has been added serves best of all, the growth appearing as a feathery track along the course of the needle below the surface. After 14 days the gelatine is liquefied. This liquefaction spreads slowly, causing disappearance of the radiating processes of the growth, until the whole of the gelatine is transformed into a cloudy glutinous liquid, at the bottom of which lie the bacilli. Growth is possible between 15°C. and 52°C. At 22°C. the bacillus develops and produces its poison slowly in gelatine, but more rapidly in Agar-Agar or broth ; but its full sporulation will not occur nor its poisons be fully produced unless a temperature about that of the body—37°C. is available. Being anaerobic, it can thus be eliminated from contaminating aerobic bacilli. Spores are rarely found in broth or in recent Agar-Agar cultivations, though they may be seen in older Agar-Agar growths even at the end of filaments of the bacilli—such filaments occasionally presenting a twisted-up appearance. Cultures of tetanus-bacilli have a characteristic foul odour due to the formation of gases ; but this odour, according to some observers, is said not to be present if the culture be a perfectly pure one, free from adventitious micro-organisms. The spores resist boiling for 4 to 6 minutes, and are very resistant to drying—facts which also serve to eliminate Tetanus bacilli from others amongst which they may be found. They can resist Carbolic Acid in 5 per cent. solution for 10 hours, although the addition of ·5 per cent. Hydrochloric Acid causes their destruction in 2 hours. Similarly, 1 in 1,000 Perchloride of Mercury solution kills them only after 3 hours, but if ·5 per cent. Hydrochloric Acid has been previously added, the time required is only 30 minutes. Air and sunlight destroy the bacilli, and probably weaken the spores ; and artificial light is said to have a similar though far less powerful effect.

Distribution.—In Nature, the Tetanus bacillus is found in the upper layers of the soil, and in the fæces of animals, but usually does no harm (for the reasons given above) unless introduced into a wound—as may happen in military surgery on active service, or in grooms, country labourers, etc. In the body, the bacillus is strictly limited to the site of inoculation, and is only present there in minimal quantities—its action being due to the poison it produces—disappearing after 24 to 36 hours, except from the pus in the wound.

Koch's Canons.—No. I. is readily satisfied. As to No. II., difficulties occur, owing to various contaminating aerobic bacilli, in obtaining a pure culture ; and also because of its own anaerobic growth and the difficulty in separating it from other contaminating anaerobic bacilli. *Kitasato's Method* for doing this is by keeping the cultivation on anaerobic plates at 37°C. for

24 hours and then boiling for three minutes, by which means other anaerobic bacilli and the full-grown Tetanus bacilli are destroyed, but as sporulation has occurred in their case their spores are able to develop into a pure culture of the micro-organism on cooling. In No. III., susceptible animals are found in man, the horse, mouse, rat, and especially the guineapig. Rabbits are fairly resistant, and dogs and birds practically immune; while amphibians are nearly so, though by raising its temperature a frog can be artificially made to take the disease.

Inoculation Experiments. Either (*a*) a virulent tetanus-culture, or (*b*) the toxins alone, or (*c*) the virulent tetanus bacilli only, or (*d*) ordinary garden-earth will reproduce the disease on inoculation into a guineapig or white mouse; but an injection of Tetanus-spores only (obtained either by filtering a culture through a Pasteur filter, or by temporarily boiling, so that the full-grown bacilli and toxins are destroyed, but not the spores) is not effectual. Further, if instead of the virulent bacilli mentioned in (*c*) above, a very young culture, or an old culture from which the poisons have been washed away, be used instead, it is a very difficult thing to reproduce the disease; for since such bacilli only form their poison very slowly, they are themselves destroyed by leucocytes or defensive substances in the blood before they have had time to produce any. An animal which has been successfully inoculated develops tetanic convulsions which first occur at or around the site of inoculation, but soon become general; and death supervenes after a short interval of hours.

The inoculation of Tetanus-spores may be rendered effectual (*i*) by distracting the attention of the leucocytes, either by combining the spores with the toxin or with Lactic Acid, or by mechanical means, *e.g.*, enclosing the spores in a pellet of Agar-Agar before inoculation; (*ii*) by mixing the spores with sand or with non-pathogenic bacilli; and (*iii*) by inoculation of enormous quantities of the spores.

Question of Infection with Tetanus.—Vaillard considers that the presence of toxins or of certain contaminating bacilli are essential for tetanus infection to occur; basing his views on the comparison of the effects of the injection of Tetanus bacilli and their toxins ($\frac{1}{10000}$ c.c. being fatal to a guineapig) with injection of the washed bacilli (the bacilli thus obtained from 20 c.c. of a culture not being fatal to a similar guineapig); and on comparing experiments with boiled cultures with those in which unboiled cultures were used. The fact of contaminating bacteria being necessary is, however, very much disputed and possibly incorrect, though it cannot be denied that the development of Tetanus is favoured by sepsis.

The Toxins of Tetanus.

Since the conditions under which Tetanus bacilli exist in the tissues at the site of inoculation are distinctly unfavourable to them because of the oxygen contained in the blood, the poison they produce must be extremely powerful. It is also formed with exceedingly great rapidity, for it has been shown that if mice were inoculated at the root of the tail and the tail itself excised in 10 to 20 minutes afterwards, yet this delay was too great and it was too late to save the life of the animal which, after a little longer incubation period than ordinarily, developed typical tetanus and died of the disease. The toxin circulates in the blood, so that the blood of diseased animals is fatal to others ; and it is even found in the tissues.

Two substances have, so far, been separated out from this toxin by Brieger who has named them "Tetanin" and "Tetanotoxin." Heat destroys the toxin far more easily than the spores, 60°C. prolonged for 5 to 7 minutes being sufficient. Sunlight, air, and artificial light are also fatal to it. The action of the poison spreads from the site of inoculation to the central nervous system, along which it ascends.

Preparation of Tetanus Toxin.—This is easily done as the toxin dissolves in water. It is best prepared by growing Tetanus bacilli for 2 to 4 weeks in broth at 37°C. by which means a most virulent form is developed so that ·000005 c.c. will kill a mouse. To preserve it ·5 per cent. Carbolic Acid is added, and the toxin kept in a cool dark place.

Tetanus—Antitoxin—Immunity.

Kitasato produced temporary immunity in rabbits by injecting a small quantity of Terchloride of Iodine along with a dose of the filtrate from a tetanus culture. The blood of such a rabbit was found to confer immunity to mice, ·2 c.c. being sufficient, and the blood of mice immunised in this way yielded similar results. On the large scale tetanus antitoxin is prepared similarly to, though of course more slowly than Diphtheria antitoxin (since the toxin of Tetanus is so extremely powerful)—horses and dogs being used. The antitoxic serum can be precipitated by alcohol from the blood of such an animal, dried, and kept indefinitely; so as to be sent by post to other towns and countries.

In dealing with mankind this serum is apt to cause disappointment, but simply because the poison is produced so rapidly by the bacillus that cases cannot come under treatment early enough. Good results have been recorded in animals, and one or two in mankind, so although the disease is only marked in man when the convulsive spasms have come on the antitoxin ought invariably to be tried since we know of no other cure for the disease.

MALIGNANT ŒDEMA.

A bacillus found, like that of Tetanus, in the superficial layers of the soil, especially if decomposing matter is present, is the cause of Malignant Œdema, which has been observed in mankind in cases of severe wounds, compound fractures, etc.

Morphology. Motile bacilli, 3μ long and $\cdot 8\mu$ broad, occurring singly, in pairs, or in small chains, with rounded ends, and possessing flagella. They can be stained by ordinary aniline dyes, but not by Gram's method. Their spores only take up colouring matter with great difficulty.

Conditions of Growth. The best temperature for its cultivation is 37°C., and when grown in gelatine to which 2 per cent. grape sugar has been added, liquefaction begins at the bottom of the tube, where this bacillus flourishes best, because it is anaerobic. It can also be grown on blood serum and on Agar-Agar; and during its growth foul gases are produced, which are said to be ignitable. Under certain conditions spore-formation takes place.

Inoculation Experiments. Susceptible animals are found in nearly all domesticated ones except cattle: but the disease is only accidentally met with in men and horses. When injected straight into the blood the bacilli are not fatal; but if a pure culture be inoculated under the skin local œdema results and is followed by general intoxication. If earth be hypodermically inoculated in a guniea-pig, in about 18 to 20 hours local œdema and subcutaneous emphysema occur, death ensuing in from 24 to 48 hours; post mortem the bacilli are to be found in the serous fluid of the œdema.

An animal which has recovered from an attack of this bacillus is said to be immune and its serum to confer immunity on other animals. This can also be brought about by injection of filtered cultures of the bacilli, but many inoculations are necessary in both cases—one daily, for eight days or so.

RABIES.

The micro-organism of Rabies is as yet undiscovered, though there seems to be no doubt that one exists. There is, however, a "virus" capable of being extracted from one animal and given to another, either from the saliva, peripheral nerves, spinal cord, or bulb. This virus is found not to spread by the blood or lymph, but by peripheral nerves to the central nervous system and thence to other peripheral nerves. In some cases it is found only in the central nervous system or in the peripheral nerves of one side—that of the wound. The passage of the virus has been interrupted, experimentally, by section of afferent nerves, thus showing that action is only manifested after it has reached the nerve-centres. The method of injection is almost invariably a bite.

The incubation period varies with the position of the point of inoculation *i.e.*, the wound, but averages on the whole about six weeks. If introduced into the subcutaneous tissue or in a region far removed from the central nervous system, *e.g.*, the foot, the disease takes much longer to develop than if it be inoculated more deeply into the tissues or in a spot nearer the nerve-centres, *e.g.*, the face, because the virus has further to travel. Even when it is injected under the dura mater directly, symptoms of the disease are not made manifest until after a lapse of 14 to 20 days—depending moreover on the strength of the virus.

Susceptible Animals are found in man and most herbivora. Cats are also susceptible as well as dogs, and bite more dangerously : and it is probably by means of the cat and dog fighting that the virulence of the virus is kept up : for the dog itself is fairly resistant, and the incubation period becomes longer and longer if the virus be passed through a series of dogs only, until at last the disease dies out altogether. Rabbits, on the other hand, are extremely susceptible, and by passages through them the incubation period can be reduced from 15 to 8 days.

Symptoms in Man.—The wound becomes reddened, is painful, and may suppurate slightly as soon as the variable incubation period has ended. Sensation of extreme dread (which passes on to excitement and perhaps delirium) follows, with convulsions which are almost invariably tonic—rarely clonic—in character ; and death occurs by spasm of the respiratory muscles. During the convulsive stage the soft palate becomes paralysed and difficulty of deglutition marked.

Symptoms in the Dog are, chiefly, the characteristic look in the animal's eye : paralysis of the vocal cords—with hoarse bark ; paralysis of the jaw with saliva hanging in festoons from it, etc.

Test of Rabies in the Dog.—This may be ascertained for certain by aseptically inoculating part of the animal's spinal cord or bulb into the eye or deep into the muscles of the neck, or (best of all methods) subdurally, in a rabbit.

Course in the Inoculated Rabbit. For four days the rabbit continues apparently well, but a rise of temperature occurs on the fourth evening. On the fifth and sixth days the temperature reaches 103° or 104°F., respiration is quickened, polyuria occurs, and the body-weight decreases. On the eighth day the rabbit appears extremely ill, and paralysis sets in, death ensuing on the tenth or eleventh day. Post mortem the nerves and central nervous system are found affected with the virus, and also the saliva. In fact, the symptoms of poison in the bulb coincide with the arrival of the virus in the saliva. If saliva be used for inoculation purposes it must be introduced under the surface of the skin and not under the membranes of the cord, because it con-

tains numerous bacteria of an infective nature. The salivary glands and the pancreas also contain the virus—as can be shewn post mortem.

Nature of the Virus.

When an emulsion of the cord of an infected animal is filtered through porcelain or heated for a short while at 100°C. or at 60°C. for 3 hours, its virulence is entirely lost. This certainly points to the supposition of some living micro-organism being the real cause of the disease and producer of the virus.

Exaltation of the Virus is brought about by passing it subdurally through a series of rabbits, a maximum being reached, at which it remains as a "fixed virus," at the 317th passage.

Attenuation of the Virus can be obtained by toning down the fixed virus by heat, or by drying—as in Pasteur's method.

Pasteurian Inoculation.

(*i*) An exalted virus, prepared as mentioned, and (*ii*) an attenuated virus, are required. This attenuated form is prepared as follows:—The "fixed" spinal cord of an inoculated rabbit is suspended in a sterilised bottle over Caustic Potash in the dark at 22° to 25°C. As the cord dries the virus becomes less virulent, and the degree of virulence can be determined by the number of days the cord has been drying. Emulsions of cords of different dates of drying are made separately in bouillon (5 c. grm. of cord to 2 c.c. of sterilised bouillon) and ɱ i—ɱ iii of one emulsion subcutaneously injected, with aseptical measures, into the flank. By this means the emulsion gets into the blood: the white blood corpuscles, and tissues generally, become accustomed to the virus while it is travelling up the nerves, and so by the time it reaches the central nervous system they have been rendered immune against it. The cords (emulsions) are used in an ascending series, from those of the fourteenth to fifteenth day of drying to those of the second to third day. This method takes sixteen days for its completion, and is known as the *Simple Method*; and if tried on a healthy dog will render the animal immune against rabies. For face-bites the *Intensive Method* is employed; a cord of only 3 days' drying being used on the sixth day, and the treatment being continued with 2 days' intermission until the twentieth day. Pasteur classified his patients into three groups: *I.*—Those bitten by animals which have been proved by growth of the virus to be mad. In these the percentage of deaths averaged about 4 only. *II.*—Those bitten by animals certified as mad by a veterinary surgeon. *III.*—Those bitten by animals considered by ordinary people to be mad.

Now, since when an animal is inoculated experimentally with rabies virus fourteen days elapse as the incubation period before the disease manifests itself, it follows that any patient who dies during treatment or within fourteen days of its completion, is not

to be included under the statistics of death, since that is due to the effects of the bite solely, and not to treatment.

Treatment of a Rabid Bite.—The vitality of the virus is destroyed by 5 per cent. Carbolic Acid solution or by Tincture of Iodine in 15 minutes, and it has already been shown that a temperature well under boiling point is also fatal to it. This latter fact is important to remember, for in cauterising a rabid bite with a hot wire it is not necessary to make the wire red hot.

As to immediate treatment, some good might possibly be done by shutting off the circulation from the limb by ligature, and heating the limb in a vapour bath ; but this latter is not practicable in most cases. As the virus can travel up the nerve-trunk at the rate of 1 c.m. in a few minutes, the hot wire should be applied within 5 minutes, but even if the case be seen later, it should be applied, as 30 per cent. of cases have recovered in which this was done up to half an hour, and in a few cases in which 24 hours were first allowed to elapse the incubation was slow. This is well worthy of notice since it affords time for the patient to apply for Pasteurian Treatment.

CHAPTER VII.

PROTOZOA.

THE MALARIAL PARASITE.

THE cause of Malaria is a parasitic micro-organism, discovered by Laveran, consisting of a pale hyaline body, with or without dark pigment granules within it, found in the blood of man in connection with malarial attacks and in no other disease.

Morphology. There are different forms of the parasite occurring at different stages of one particular variety of malarial fever, and also in different varieties of the fever. Chief amongst these forms are the following:—(*i*) Minute nucleated bodies found free in the blood; (*ii*) small bodies which lie either on or within the red corpuscles, possessing amœboid properties and multiplying by fission. Later on, in a Tertian Ague attack, they enlarge, contain pigment, and merge into the form *vi* (*b*); (*iii*) large pigmented intracorpuscular bodies, which may multiply by fission; (*iv*) mulberry-shaped intracorpuscular forms, called "rosette-bodies," composed of about 15 to 20 spherules circularly grouped round a number of centrally-placed pigment granules, and probably representing the principal mode of multiplication of the parasite in the blood. They are seen best at the beginning of the rigor of an attack of Tertian Ague, or even before the rigor begins. These rosette-bodies later on escape out of the red corpuscle, and after being found free outside it in the blood give rise to (*v*) Spores, which are found free and derived from the breaking up of the rosette-bodies outside the corpuscle; and (*vi*) various intermediate forms which make up the life-history of the parasite either within the blood or outside the human body. Most important amongst these are—(*a*) an intracorpuscular hyaline crescent, containing pigment granules, which is not important within the circulating blood, but when outside the body assists towards the development of the parasite. This form can be seen in the blood for days and weeks after the malarial fever has subsided and the other forms are no longer seen. It does not change its shape within the body, and is not attacked by leucocytes; and (*b*) a nucleated finely pigmented intracorpuscular form, developed

from (*ii*), which enlarges and becomes actively amœboid at the end of a tertian ague attack, but later on, some hours before the next attack is due, seems to lose its vacuoles and develop coarse pigment granules within it, which later on arrange themselves in a cluster. Possibly, just at the beginning of the attack, this form of the parasite becomes converted into the "rosette-body."

The cycle of the Malarial Parasite within the blood of man is considered to be, in its main points, as follows:—The large intracapsular form is the mature micro-organism, and as its sporulation occurs it passes into the morula-body which breaks up into the small spheres found free in the blood. These first attach themselves to red corpuscles, then, entering them, begin to grow, each at the expense of the hæmoglobin of its own corpuscle, which it absorbs and converts into pigment. Later on, becoming enlarged, they eventually reach the stage of the large pigmented amœboid intracorpuscular forms which started the cycle.—(Manson.)

Why does the Malarial Parasite attack man?—It is acknowledged on all sides that the presence of man is not necessary to the life of the parasite, but that it can live and multiply quite independently of him; for, on the one hand, in the tropics, malaria abounds in places where man is seldom or never seen, and on the other hand, there are districts where hardly a human being exists who is not at some time or another the subject of malaria. The parasite apparently attacks mankind with a definite purpose in view—probably because it finds a more suitable soil for its development at certain stages of its history in the human body than elsewhere. How it probably leaves him and returns to him, as to a host, we shall see later on.

Life of the Malarial Parasite outside the human body. (Manson). *I.*—If malarial blood be left, in a microscopical preparation, for some 20 to 30 minutes after it has been drawn from a patient and then examined, a peculiar flagellated form of the parasite is seen. As this form is not seen directly after the blood is shed from a malarial patient, it does not exist within the circulating blood. Its derivation is found to be from two sources—(*a*) at certain times, in tertian and quartan agues, the large pigmented intracorpuscular amœboid forms (*iii*) are seen to squeeze themselves out of the red-corpuscles. This occurs not immediately but some time after the blood has been taken out of the body. They then assume a spherical shape from which flagella are afterwards thrown out—bursting through the periphery of the sphere. After a time the flagella become detached and fall away from the sphere. (*b*) In some malignant varieties of malarial fever the crescentic form of the parasite, which appears to be unimportant in the circulating blood—as it exists for some weeks after the fever has ceased—is seen to undergo certain changes after the blood has been shed. It

first becomes straightened : next, assuming first an oval and then a spherical form, the pigment within it becomes collected and then scattered and undergoes oscillatory and other movements, while the sphere itself takes on amœboid properties and changes its shape. Later on, flagella burst through the periphery of the sphere, and having remained attached to it for some time become separated from it.

The movements of the flagella, both when attached and when free, are of two kinds—(*i*) rapid curling motion ; and (*ii*) a shivering vibration, during which the flagella remain almost or quite straight.

The flagellated body is the first stage of the life of the malarial parasite outside the human body, corresponding to the morula form found within the blood ; and the flagella constitute the second stage, as will be discussed further on. The central sphere is only a residuum of the whole process.

Behaviour of the Leucocytes towards the Malarial Parasite.—So long as the parasite is within a red-corpuscle, phagocytosis does not occur, although certain leucocytes can be seen to "try" the red corpuscle, so to speak, as if there were something amiss within, but they never envelope it—the ensheathing red corpuscle apparently guarding the parasite from the hostile intention of the phagocytes. When, however, the parasite escapes from its corpuscular envelope—as in the flagellated body seen in blood which has been withdrawn from the body—it is in a condition to be eaten by the phagocytes ; but this does not actually occur—apparently because the latter have been cooled down on the slide. Hence, for protective reasons as much as for food, does the parasite shelter itself within a red corpuscle, nor does it quit that shelter in the circulating blood, for under these conditions the phagocytes would devour it.

II.—Since the parasite has not been found in secretions or discharges, normal or pathological, from the human body, it follows that it probably does not leave the body in these liquids. Further, as hæmorrhages are extremely rare in Malarial Fevers, they do not stand for any account as a factor, either. This implies that some agency must exist without the body, by whose means directly or indirectly the parasite can regain admission into it. This agency is generally believed to be the mosquito, and in fact malarial blood containing the parasite has actually been seen within the insect itself; and from this fact the analogy between the life-histories of the Malarial Parasite and Filaria Sanguinis Hominis is distinctly marked. It is to be noted also that in India during the rainy season, when malarial fevers are most prevalent, the number of mosquitoes is much greater than at any other time of the year. The female mosquito attacks a patient suffering from malarial fever, and sucks up a small

quantity of his blood into her stomach, within which the flagellated body is developed. The flagella, when free, are said to bore their way through the stomach walls into the muscles of the mosquito, where they possibly become encysted. (The male mosquito, not being a blood-sucker, takes no part in these proceedings.) The female mosquito then retires to a tank or other body of water, deposits her eggs and dies, falling into the water herself. Larvæ are developed from these eggs and lie floating on the surface for a time and later give rise to the young mosquitoes. As the dead body of the parent, with its inbred malarial parasites, lies in the water, man on drinking it becomes liable to be infected with malarial disease ; or again, if the tank or pond dries up, the desiccated parasites become blown about, inhaled by man, and in this way may communicate malaria to him.

Behaviour of the Parasite within the Mosquito (Ross).—As described above, soon after they have gained entrance into the stomach of the mosquito, the crescentic forms become converted into spheres, and most of these in half-an-hour become flagellated bodies, though some of them are eaten by phagocytes. The flagella, later on set free, are said to perforate the wall of the stomach and enter the muscles of the mosquito where they become encysted.

Experimental administration of Malaria was performed by Ross, by giving patients water, in which mosquitoes had died after depositing their eggs, to drink.

Arguments against the Mosquito Theory are based on two facts—(*i*) Mosquitoes abound in places where there is no malaria—but this is really no objection—and (*ii*) Malaria exists in places where there are no mosquitoes. It is quite possible, however, that it may have been conveyed thither by wind, infected water, etc.

Lawrie's Theory.—Surg.-Lieut.-Col. Lawrie, of Hyderabad, lately denied the existence of the Malarial Parasite, stating that the forms supposed to be recognised as such in malarial blood are nothing more or less than either—(*a*) unformed, or (*b*) degenerate varieties of leucocytes. He bases his opinion on certain observations he made on the development of the white cells of the blood and on the behaviour of the white corpuscles in the frog ; but as he makes but little reference to crescent forms, and has practically nothing to say concerning the "rosette-bodies," it is quite impossible to do more than attract attention to his theory, which in its present undeveloped condition and in the face of writings of such a large number of celebrated observers concerning the malarial parasite, requires further elucidation before full credence can be attached to it, or itself raised to the position of being regarded as an argument against the generally accepted opinion that the "malarial parasite" is the cause of malaria.

CANCER.

In certain forms of cancer a micro-organism is found which has been variously named Prorosperm, Cancer-body, and Cancer-parasite. None of Koch's Canons are fulfilled with regard to it, but, although there is no definite proof that it is due to this parasite, or that the parasite itself is a protozoon, the possibility of cancer being a parasitic disease is undoubtedly strong.

Morphology. The "cancer-body" is found as a spherical refractile mass in the protoplasm of epithelial cells (and some say in their nuclei also), and consists of a central ovoid or irregularly shaped portion embedded in a surrounding protoplasm which almost or quite fills up a double capsule. These bodies increase in size within the epithelial cell, flattening out its nucleus, and are said by some to possess amœboid movements and to multiply by division. They have never been cultivated and inoculation of cancerous tissue on animals has hitherto always been negative in its results.

AMŒBA COLI.

A protoplasmic mass, with a nucleus, granules, and vacuoles, possessing amœboid movements and powers of assimilation. It is found in the stools and ulcerations in the intestines in Epidemic Dysentery: also in the Liver Abscess which is consequent on that disease. Cunningham attaches no importance to this amœba, having also found it in cholera and other diseases, and in the normal contents of the intestine of the horse and cow. Lösch, however, gave dogs some fresh human dejecta containing these amœbæ and in one case recovered them from a slimy mass passed per rectum; this dog died and typical dysenteric ulceration was found in its intestine.

CHAPTER VIII.

MOULDS.

ACTINOMYCOSIS.

Occurrence.—The Ray-fungus or Actinomycosis is found in the pus of certain abscesses of the lungs, liver, and other organs of subjects of the disease ; also in bones, especially of the spinal column and jaw (in cattle) ; and in the urine and fæces when the kidneys and alimentary canal are affected.

Morphology. It is found in the centre of hard fibrous nodules as soft caseous masses, varying in size from a tiny pin's head to a hemp-seed, and is contained in a sort of reticulum situated within a capsule. Microscopically each mass is seen to consist of three zones ; an outermost, composed of club-shaped wedge-like rays with rounded bases, appearing to be set on star-fashion when viewed in section, and surrounded by giant-cells and others which often contain small portions of the parasite in their substance ; an innermost, consisting of "cocci" in chains ; and a middle zone made up of branching mycelial threads of leptothrix which pass from the centre to the periphery. The cocci are $\cdot 5\mu$ in diameter. The threads, somewhat larger, vary in length, and are either split up into short rods or not divided at all. Near the margin they branch, and at their extremities the cocci are often found swollen out into a club-shape. The threads are the active portion and capable of artificial growth ; but the "clubs" do not develop into anything. Sections of the growth in tissues can be stained by Gram's or Weigert's Method.

Conditions of Growth. Artificially cultivated at 37°C. on blood serum fine granules are first formed which enlarge and form a filmy layer, cocci, threads, etc., being detected under the microscope. On agar-agar or glycerine-agar hard nodules are to be seen. The best results are obtained by anaerobic cultivation.

Inoculation Experiments. Susceptible animals are found in cattle, pigs, rabbits, and man. The typical disease can be reproduced by peritoneal inoculation in rabbits, or subcutaneously in calves. It may spread within the body by embolic metastasis.

Existence outside the body may occur on straw or cereals. Actinomycosis enters the body by wounds and scratches, and perhaps through carious teeth (cattle) or the tonsils (man, pig).

Nature of Actinomycosis.—While usually regarded as a fungus, some say the micro-organism is a bacillus, the central portion being composed of bacilli and spores.

MADURA FOOT.

A chronic disease, mostly of the feet, sometimes of the hands, caused by the entrance of a mould through a skin abrasion or wound, which affects all the tissues indifferently. Found in India, especially in Madras. Men of the agricultural class are chiefly affected, and usually between 15 and 40 years of life. After about a couple of weeks' incubation period the disease begins insidiously, and later on a nodule, whose deep attachments are ill-defined, appears under an indurated adherent discoloured skin. The ball of the great toe or the soft tissues at the bases of and under all the toes are the chief spots that are first invaded. The disease progresses slowly, swellings of variable size appearing in the course of months, and after 12 to 18 months or so these break down, ulcerating through the skin and forming sinuses which discharge seropurulent matter containing pinkish or black bodies. According to the nature of these small granules discharged a "white or pale" and a "black or melanoid" variety of the disease is recognised.

Morphology. This mould differs from Actinomycosis in not spreading by embolism, and in affecting external surfaces. Occasionally, its hyphæ have been seen piercing the wall of an artery and existing within its lumen. It stains with Gram's method, and consists morphologically of branching spore-bearing hyphæ much resembling the Ray-fungus.

Conditions of Growth. Obtained from the "white variety," growth occurs slowly on Glycerine-Agar at 37°C. with the formation of colonies which are flat and colourless at first, but later on become pink at the periphery and depressed at the centre. These colonies are of hard consistence and rarely run together and coalesce. They appear much like the ova of the frog. Growth can also be obtained on potato, and is aerobic.

Inoculation Experiments in animals, as a rule, produce only local irritation. Very few giant-cells are found in connection with a "mass," which consists of mycelial threads in its central portion.

FAVUS.

A parasitic disease of the skin, chiefly of the hairy scalp, caused by a fungus named *Achorion Schœleinii* and consisting, morphologically, of a mycelium branching at right angles and short tubes with conidia-spores. Growth begins in the hair-follicles, passing into the hair-sheath and surrounding epidermis, separating and destroying the cells and hairs. The cutis vera is not directly implicated, though inflammation may occur in this part through irritation from the crusts formed by the disease. These crusts have a peculiar mouse-like odour.

Conditions of Artificial Growth. On gelatine plate-cultivations white colonies are formed with liquefaction of the medium at their margins. On a gelatine slant-tube a yellowish membrane slowly forms, with liquefaction of the gelatine. On Agar-Agar the growth is white and adheres firmly to the medium, no liquefaction of which occurs.

THRUSH.

Greyish-white spots on the mucous membrane of the tongue and mouth—seen mostly in children, but also met with in adults—which may spread to the pharynx and œsophagus, are seen in this disease, and are due to the mycelial threads and spores of the fungus.—*Oidium Saccharomyces Albicans.*

Growth first occurs in the centre of the mucous membrane and extends both to the surface and to the deeper parts. The cells are round, oval, or cylindrical ; these latter are many times as long as they are thick, so that, applied end to end, they practically form filaments at the extremities of which the smaller cells are found. Spores form in the round cells.

Artificial Growths. The fungus has been cultivated on plates as white colonies, and is said to be able to ferment sugar. Artificial inoculation of this disease has been successfully performed in the vagina. The Thrush-fungus is pathogenic in rabbits, and after inoculation long mycelial threads are found in various organs.

RINGWORM.

Three forms of this disease exist, known according to the portion of the body attacked On the head it is called "Tinea Tonsurans"; on the chin "Tinea Sycosis"; and on parts devoid of hair, *e.g.*, the body, "Tinea Marginata." The fungus, *Tricophyton Tonsurans,* has a short-threaded mycelium, and consists chiefly of spores, which infiltrate the hair-follicle sheath and shaft and surrounding epidermis—separating and destroying the cells.

Conditions of Artificial Growth.

In plate cultivations colonies form quickly, with liquefaction of the media. In gelatine a membrane, white and powdery above, and yellowish underneath, is formed.

Susceptible animals are found in dogs, horses, cattle (especially calves), and man, and it is transmissible from animals to the human being, and *vice versâ*.

YEASTS.

The itching of the genitals in Diabetes in due to an eczematous condition caused partly by the condition of the urine *per se*, but chiefly by the irritation produced by the growth of *Saccharomyces Cerevisiæ*, a yeast whose characters have already been described under the general heading of its class in Chapter I.; and perhaps also to a less extent by a mould—*Penicillium Glaucum*.

CHAPTER IX.

ON STAINS AND STAINING METHODS.

THE stains commonly employed in bacteriological work are the following :—

I.—**Methylene Blue**—concentrated aqueous solution.

II.—**Gentian Violet**—concentrated alcoholic solution.

III.—**Fuchsin**—concentrated alcoholic solution.

The above are simple aniline basic dyes, requiring filtering before use, and are convenient for staining cover-glass preparations of micro-organisms. It will often be found more satisfactory to use a somewhat diluted solution instead of a concentrated one. The cover-slip on which a film of the micro-organisms has been spread is gently dried ; fixed by being passed (held—smeared surface upwards—in the fingers so as to prevent over-heating) three times through a Bunsen or a spirit flame ; cleared—if necessary—of its ground substance by immersion in weak acid (*e.g.*, ·1 or ·2 per cent. Acetic Acid in water) for a few moments ; washed in distilled water ; redried in the flame ; next placed—face downwards—in the staining solution for 1 to 5 minutes ; washed, dried, and mounted in Xylol Balsam.

When required for Gram's method of staining (see below) 11 parts of the above Gentian-violet solution are mixed with 100 parts of aniline water (4 of aniline oil to 100 of distilled water).

IV.—**Eosine Solution.**—·5 per cent. of Eosine in 50 per cent. alcohol. Useful for staining the eosinophilous granules of leucocytes, red blood corpuscles, or tissue ground-work in double staining of sections.

V.—**Loeffler's Methylene Blue** stains many micro-organisms better than the ordinary solution of methylene blue, and can be used in most cases. Its composition is—Concentrated Alcoholic Solution of Methylene Blue, 3 parts, to Caustic Potash Solution (1 in 10,000) 10 parts.

VI.—**Carbol-fuchsin** (Ziehl) consists of—Concentrated Alcoholic Solution of Fuchsine, 11 parts, to 100 parts of 5 per cent.

Aqueous Solution of Carbolic Acid. It requires filtering before use. An extremely convenient stain for spores. The film of the micro-organism having been fixed on the cover-glass as described above, is left in weak (5 per cent.) Chromic Acid Solution for a couple of minutes, washed in distilled water, dried, and placed in warm Carbol Fuchsin Solution for 5 minutes (or the stain may be poured on the specimen which is held lightly over the flame of a spirit lamp at such a height that steam only just begins to come off, more and more staining solution being added as evaporation proceeds, for a few minutes). The cover-glass is then washed in distilled water, the specimen decolorised in 20 per cent. Sulphuric Acid for 20 to 30 seconds, immediately washed in water again, and finally counterstained in Methylene Blue. The spore appears red and the rest of the bacillus blue.

VII.—**Gram's Method.**—A cover-glass film is prepared and dried in the flame and then fixed by immersion in alcohol for a few minutes. It is next transferred to a Gentian-violet solution (see above) for 30 to 50 seconds ; excess of stain soaked up with filter-paper ; and the specimen placed in Gram's Iodine Solution (Iodine, 1 grm. ; Potassium Iodide, 2 grms. ; Distilled Water, 300 c.c.) for 30 to 50 seconds, by which means the film is turned blackish. After the excess of the Iodine Solution has been soaked up, the coverslip is carefully washed in absolute alcohol until no more Iodine seems to come away, then in distilled water, and finally dried and mounted in Xylol Balsam.

This method is most useful for many micro-organisms, staining them a dark brown colour. It is perhaps the best way to stain pus, the pus cocci and nuclei of the leucocytes being rendered violet, while the ground substance and the protoplasm of the leucocytes appear pinkish-yellow. Sections containing micro-organisms can also be stained in this way—*cp.* Leprosy, Actinomycosis, etc., care being taken in clearing the specimens. Some micro-organisms, however, will not stain by Gram's Method—*e.g.*, Koch's Comma-bacillus, the Bacilli of Glanders and Malignant Œdema, Friedlander's Bacillus, Neisser's Gonococcus, Spirillum Obermeieri, and the Typhoid Bacillus.

VIII.—**Weigert's Fibrin Stain** is extremely useful to bring out the fibrin network in Lobar Pneumonia, and also for staining sections. The specimen having been stained in Picrocarmine and placed on a slide, a few drops of Anilin-gentian-violet solution are poured on to it and allowed to remain a quarter of an hour or more. Excess of stain having been removed with filter-paper, the specimen is washed in ·6 per cent. Sodium Chloride Solution, and a few drops of Weigert's Iodine Solution (Iodine, 1 part ; Potassium Iodide, 2 parts ; Distilled Water, 100 parts) is poured on and allowed to remain for two minutes. Excess is removed with filter-paper ; the specimen decolorised with a weak solution of Aniline

Oil (2 parts) and Xylol (1 part), thoroughly. Lastly, it is well washed in Xylol and mounted in Xylol Balsam.

IX.—**Czinziski's Solution** affords a convenient method for staining micro-organisms in tissues. It consists of Concentrated Aqueous Solution of Methylene Blue, 50 c.c.; Eosine, ·5 grm.; Absolute Alcohol, 70 c.c.; Distilled Water, 130 c.c. The section having been immersed in absolute alcohol for 5 minutes is kept in the stain for about 5 to 10 hours, then well washed in distilled water, dried, cleared, and mounted in Xylol Balsam. The micro-organisms appear blue in a pink tissue ground.

CHAPTER X.

ON THE BACTERIOLOGICAL EXAMINATION OF LIQUIDS, FLUIDS, AND SOLIDS.

EXAMINATION OF WATER.

Quantitatively. This method will afford a rough knowledge of the bacteriological state of any water, whether obtained from a well, tank, or river—and in these cases samples from different depths and positions must be taken—or from a tap either directly connected with the main supply or after methods of filtration, near or remote, whose efficiency can also thus be tested. Tap water should always be allowed to run for about a minute before being tested in order to procure a fairly representative sample. The specimens of water, whencesoever obtained, must be at once placed in sterilised flasks and examined as soon as possible.

I.—**Plate-culture Method.**—100 c.c. of the water, after having been thoroughly shaken, are measured off into a sterilised flask and ·1 c.c. (or less) to ·25 c.c. drawn off by suction into a graduated sterile pipette and transferred to a tube containing sterile liquefied gelatine. Three or more such tubes should be made for comparison and control. Each tube having been gently but well shaken, so as to thoroughly mix the water and gelatine together, is emptied into a labelled Petrier's dish, which is then covered over, kept in an incubator at 21°C., and examined from day to day—the colonies that appear being counted either on the whole of the surface or only on a measured part of it, according as they are few or very numerous. By this means the total number of micro-organisms in the quantity of water taken for examination is calculated, from which the average number in one or more c.c. of the sample can readily be deduced.

II.—**Roll-tube Method.**—Instead of using Petrier's dishes, the gelatine tube containing the pipette-ful of the water is placed horizontally in a groove on a block of ice, and turned round till the gelatine thickens, when it is allowed to set, and then the tube is placed in the incubator at 27°C., and the colonies that grow on the surface of the gelatine counted daily. In both the above methods Agar-Agar may be used instead of Gelatine.

Qualitatively.

I.—**Examination for the Presence of Cholera Vibrios.**—Besides the method already given under the heading of "Cholera," the following are useful :—

(*a*). *Peptone Method.*—50 c.c. of 2 per cent. Peptone Solution is placed in half-a-dozen small sterilised flasks and into each a small quantity—5 c.c., 10 c.c., etc., up to about 50 c.c.—of the suspected water is poured and gently mixed, and the flask kept in the incubator at 35° to 37°C. for 18 to 24 hours. The surface of the peptone is then examined for cholera vibrios (staining with Anilin-gentian-violet) especially wherever a film is visible ; and on these being discovered gelatine plates and stab-cultures may be made and kept at 21°C., or Agar-Agar stroke-cultures (a temperature of 37°C. being required) and the specimens examined from day to day. A peptone tube-culture may also be made and examined chemically for the red coloration (*see* "Cholera") after 18 to 24 hours' incubation at 37°C.

(*b*). *Agar-Agar Method.*—Three Agar-Agar tubes are liquefied and cooled down to 40°C. One tube is then inoculated with ·25 c.c. of the suspected water and gently mixed. From this tube the second is inoculated by means of a platinum loop dipped in three successive times ; and the third tube similarly from this second one. Plates are then prepared from these three tubes and labelled, kept at 37°C. for 24 hours, and then examined. The first plate may yield crowds of colonies of all sorts, hence the value of the second and third plates in which the colonies are more separate and their distinctive features better able to be studied. If cholera vibrios be found, gelatine stab-cultures or Agar-Agar stroke-cultures can be made, and experiments conducted on guineapigs ; or a peptone-tube can be inoculated and examined chemically for the red-coloration.

(*c*). *Gelatine Method,* by plates, as described, the incubator being kept at 21°C.

II.—**Examination for the Typhoid Bacillus and Bacillus Coli Communis.**—About 1,000 c.c. of the suspected water are filtered through a sterile Pasteur or Berkefeld filter. The "candle" of the filter is then gently scraped with a sterilised bit of ivory or brush, and this washed carefully in 20 c.c. of the filtered sterile water, which thus becomes opaque and cloudy. Of this ·25 c.c. is added to each of several tubes containing liquefied cooled carbolised gelatine (the proportion being 8 grms. of gelatine to 100 c.c. of ·5 per cent. solution of Carbolic Acid) and mixed carefully. Plates are then prepared, as above described, and kept in the incubator at 21°C. (The presence of the Carbolic Acid prevents the growth of many micro-organisms, especially of those that cause liquefaction of their medium.) The plates are examined from time to time, and on the appearance of colonies resembling

those of the bacilli under search stroke- and stab-cultures are made in gelatine, and examined for growth characteristics and microscopically. For the distinguishing points between these two bacilli, *see* "Bacillus Coli Communis."

The Examination of Ice-creams is conducted upon practically the same lines as that of water.

EXAMINATION OF MILK.

Quantitatively. The sample of milk having been thoroughly shaken is collected into several small sterilised flasks from which varying quantities of from ·1 c.c. to about ·5 c.c. are drawn up into sterilised pipettes, introduced into different tubes containing sterile liquefied gelatine, and plates, etc., prepared as described under the quantitative Examination of Water.

Qualitatively. *I.*—**Examination for the Typhoid Bacillus and Bacillus Coli Communis.**—A small quantity of the sample of milk is diluted with an equal amount of sterile distilled water and the examination conducted as described above.

II.—**Examination for Streptococcus Pyogenes and the Diphtheria Bacillus.**—The sample of milk having been stirred up, a platinum needle is dipped into it, and a 3-stroke culture made on three successive sloped Agar-Agar tubes, proceeding from one tube to the other without re-dipping the needle into the milk. The tubes are kept at 37°C. and investigated after twenty to twenty-four hours, suspicious colonies microscopically examined, and further cultures made on sloped Agar-Agar, or (in the case of the Diphtheria Bacillus) on sloped alkaline Serum Agar.

III.—**Examination for Tubercle Bacilli.**—To 50 c.c. of the suspected milk in a sterile flask 10 c.c. of strong liquefied Carbolic Acid are added. The mixture is shaken for five minutes, and then poured into a conical glass and allowed to stand, covered over and protected from dust or other contamination, for twenty-four hours, at the end of which time a little of the deposit at the bottom is removed and cover-glass specimens prepared and stained (*see* "Tuberculosis"), in the usual way, for the bacilli.

EXAMINATION OF AIR AND DUST.

I.—**By Aspiration through Sterilised Bouillon.**—About 50 c.c. of sterilised beef-broth are placed in a sterilised flask and a known quantity of air aspirated through it in a definite time, *e.g.*, half-an-hour. 1 c.c. of this mixture is then added to a tube of sterile gelatine and stirred (several such tubes being prepared), poured

on to a Petri's dish and kept at 21°C. and examined for colonies daily. Various moulds, yeasts, staphylococci and sarcinæ will be found.

II.—**By method of Plate-cultures.**—Sterile gelatine plates are exposed for different measured times (half, one, or two hours, respectively) at different heights and positions in a room or building ; they are then covered over and kept at 21°C. in an incubator and examined daily. Plates placed nearer the ground or in a draught will shew more growths than those placed higher up or where the air is not circulating so freely ; while greater length of exposure will cause an increase in the number of colonies found.

III —**For Anaerobic Micro-organisms.**—Air is aspirated through melted gelatine in a wide-mouthed flask or tube. The gelatine is then allowed to set slantwise, and the mouth of the tube accurately fitted with a doubly bored cork. Through one bore, a piece of glass piping, reaching nearly down to the bottom of the vessel, is connected with a hydrogen gas apparatus, and through the other bore, a short tube which serves for an outlet for the gas, is fixed. In this way all the air can be expelled from the vessel and replaced by hydrogen gas, and anaerobic colonies cultivated. The hydrogen should be passed through solutions of Lead and Silver Nitrate (1 in 10), and Pyrogallic Acid in 1 per cent. Caustic Potash Solution, in order to remove traces of Sulphuretted Hydrogen, Arseniuretted Hydrogen, and other impurities.

EXAMINATION OF EARTH AND SOIL.

Micro-organisms exist freely on the surface of the soil, but few are found at a depth of 3½ to 4 feet, and none are present at over 6 feet from the surface. As soon as a specimen has been collected, it must be examined.

I.—**Ordinary Examination of Surface Soil.**—Minute portions of the surface-soil are mixed with sterilised liquid gelatine, and plate cultures, roll-tubes, etc., prepared in the manner already described above.

II.—**Examination for Anaerobic Bacilli.**—Gelatine roll-tubes having been prepared with earth taken from below the surface (there are various instruments devised for doing this without contaminating the specimen) are loosely plugged and placed in a wide-mouthed bottle which is connected with a hydrogen apparatus, and the air is displaced by that gas. The tubes are examined daily.

III.—**Special Examination for Tetanus Bacilli and Bacilli of Malignant Œdema.**—In some of the tubes (see *II.*) typical colonies of these micro-organisms may be seen, in which case grape-sugar, gelatine or Agar-Agar cultivations are made from them, heated to

60°C. for 30 minutes, and then kept anaerobically for 24 hours; re-heated and placed in the incubator—this process being repeated as often as is necessary. The Tetanus Bacillus and the Bacillus of Malignant Œdema, being very resistant, will survive when the other anaerobic bacilli are killed by the heat. Experimental inoculations into animals will greatly assist their isolation.

EXAMINATION OF SUSPICIOUS AND UNSOUND MEAT.

I.—Portions are given to rats for food, and if they die plate and stroke cultures are made from the spleen, liver, lymphatic glands, lymph, serous fluids, and blood taken from the heart. The colonies that appear are examined microscopically and further cultivations made from them.

II.—A portion is minced and an extract made with sterile beef-broth. Rats, guineapigs, etc., are inoculated subcutaneously with some of this extract, and on their death their organs and blood are examined.

III.—Gelatine plate cultures are made with small portions of this bouillon extract, and the colonies obtained are microscopically examined, and grown.

IV.—Portions of the bouillon extract are mixed in liquefied sterile gelatine tubes which are then kept anaerobically (see above) and further inoculations made from the growths so obtained.

BACTERIOLOGICAL EXAMINATION OF A DEAD ANIMAL.

The hair on the chest and abdomen is shaved off, and the skin well washed first with 1 in 1,000 Mercuric Chloride and then with Absolute Alcohol, and carefully reflected with sterile instruments by means of crucial incisions. Next the median and transverse lines are cauterised with a red-hot iron and the chest and abdomen opened with sterile instruments. Gelatine plates and Agar-Agar stroke-cultures are made from the local lesion, the spleen, heart's blood, peritoneal fluid, etc., and kept at 21°C. and 37°C., respectively, colonies being watched for and further examination conducted as already described. Cover-glass preparations of the spleen, blood, peritoneal fluid, etc., are also prepared at the same time, stained with Loeffler's Blue and examined for micro-organisms.

CHAPTER XI.

Remarks on the production of Immunity by Prophylactic Inoculations and the treatment of Bacterial Diseases by Serum-Therapy.

THE further the bacteriology of diseases produced by pathogenic micro-organisms is studied, the more does their classification, as given above, into Septicæmias, Local Processes, and Intoxications hold good, but at the same time the more have Local Processes been found to merge into Septicæmias by reason of the discovery of their micro-organisms in the blood. Thus, we find that Enteric Fever, which formerly was regarded as a Local Process by most authorities and as an Intoxication Process by a few, is now looked upon by many as a Septicæmia ; and Bubonic Plague also seems to occupy a similar position. On the other hand, the only two diseases actually known as Intoxications are Diphtheria and Tetanus, although Cholera must also be so regarded since the separation of the toxin of Cholera from its bacillus is a very difficult matter, and it is doubtful if it has really been accomplished. Pfeiffer, in fact, states that it is not possible to obtain the toxin of Cholera in the filtrate of a bouillon culture unless that culture be very old—when a trace will be found—and it is only present under those conditions because some of the vibrios have died and in the act of dying set free their self-contained poison. Through his investigations it has been demonstrated that for inoculation purposes in cholera the dead bacilli act just as well as the living bacilli ; and since dead cultures are far less dangerous than living ones, they are used for this end in Wright's Inoculation against Enteric Fever, and in Haffkine's recent inoculations against the Plague in Bombay.

We have seen that immunity can be brought about against Tetanus in animals by using filtered cultures, and in Diphtheria in mankind by the prophylactic injection of the antitoxin contained in the blood serum of immunised animals, and since in these instances no bacilli are injected, the question is at once raised : " How is this immunity brought about ? " Phagocytosis cannot occur, because there is nothing for the phagocytic leucocytes to exert their action upon, and indeed this is one great fact against the acceptance of the ingenious Theory of Phagocytosis which Metchnikoff put forward. It is now believed, however, that immunity in these cases is due to the accumulation in the blood and tissues of certain sub-

stances, called "protective proteids" (Hankin), having an antitoxic power whereby they more or less completely counteract the products by which pathogenic micro-organisms bring about their deleterious effects. These substances are the result of the metabolism of tissue cells, and can be obtained from the blood, spleen, and lymphatic glands. Leucocytes may be responsible for this result in a small way, but would certainly seem to play no important part in the production of immunity. Another point to be noted against phagocytosis is the fact that if a frog be heated to 37°—40°C. and then anthrax bacilli inoculated, no phagocytic reaction is seen to occur.

In considering the question of immunity, caution must be observed in the acceptance of any theory not backed up by facts, and as the subject is an extremely difficult one, even the view stated above, which appears to be the most recent, must not be taken as conclusive and final. Further investigation is necessary to clear up this most interesting and intricate subject.

Preventitive Inoculations and Serum-Therapy. In any vaccination against a disease our two-fold aim is—(*i*) to obtain a degree of immunity equal to or greater than that produced by an attack of the actual disease, and (*ii*) to do this without risk of life or health. Now, if we simply inoculate with the living micro-organisms or their products, we can fulfil the first condition, but not the second. This latter can be attained either—(*a*) by inoculating a patient with micro-organisms which have lost their virulence for man by being passed through certain animals—as in the present method of vaccination against Smallpox ; or (*b*) by employing artificially attenuated micro-organisms—as in Pasteur's inoculation against anthrax ; or (*c*) by using measured quantities of dead but still poisonous micro-organisms. This last method is the least dangerous and most easily gauged as to the strength of the vaccine used. Examples of it are seen in Pfeiffer's Anticholera inoculation, Wright's Antityphoid inoculation, and Haffkine's preventitive inoculation against Plague. In all these methods the measurement of the dose to be employed is the most important and difficult question, for the initial virulence of the culture taken, the exact composition of the medium on which it has grown, as well as the temperature and duration of its growth, are important but difficult points which require great exactitude. The actual amount to be given is measured by effects on animals, *e.g.*, guineapigs in the case of Antityphoid vaccines, and mice or rats in Plague ; but when applied to man the dosage is of necessity so various that many strange and almost paradoxical results (by no means wholly due to differences in the reaction and resistance of the patients inoculated) occur, and are most probably due to some defect in the cultivation of, and technique in administering the vaccine. No measurement in immunising units (as in Diphtheria Antitoxin) has been realised beforehand in these vaccinations, and consequently, some method of gauging the dose by the effect and degree of immunity it confers has to be adopted.

This can be done by Pfeiffer's method of serum diagnosis, which consists in noting the following facts :—The serum of an animal or patient now suffering or who has suffered from an attack of the particular disease is added to a pure culture of the micro-organisms of that disease, and it is seen that—(*i*) the mixture becomes turbid ; (*ii*) the bacteria become agglutinated and form masses ; (*iii*) losing their motility, if they possessed any ; (*iv*) the conglomerate masses sink to the bottom, leaving the previously turbid fluid clear ; (*v*) the bacteria shrink up into the form of minute spherules ; (*vi*) and are definitely killed. The first four of these phenomena are the most important ones. According to the degree of dilution possible so can one measure the effect of the immunising process. Now, during an attack of an infective disease, this process of immunisation is continually going on from the very first onset—that is to say, an antitoxin is continually being formed—but at the same time the bacilli are also growing and generating their toxin ; and so, although the individual may have gradually formed quite enough of the antitoxin to produce complete immunity if that amount could have been injected all at once, yet by reason of the delay in its production a fatal issue cannot be prevented In other words, a degree of immunity which would be certainly effectual if attained on the first day of the disease may be quite ineffectual if produced later on during its course.

(The practical drawbacks against these preventitive inoculations, with regard to the public, are the pain and fever caused by them. The local œdema which follows the inoculation is largely due to a marked decrease in the blood coagulability (not to the local presence of crowds of phagocytes as some suggest) and can be greatly diminished by the administration of Calcium Chloride along with the inoculation ; while excessive pain can often be relieved by lightly painting a 5 to 10 per cent. solution of Menthol over the part every hour or two)

The result obtained is often greater after inoculation than after an actual attack of the disease. Pfeiffer succeeded in cultivating cholera-vibrios on a goat's serum which exerted a sedimenting influence in much more than a 200-fold dilution, and this is also seen—though of course in a far less degree—in patients who have received preventitive inoculations against Typhoid Fever.

From these facts it has been argued that the serum of immunised animals should be utilised in the treatment of the same disease in man, and theoretically this would seem to be all correct. Practically, however, certain difficulties arise. Firstly, we have to note whether the serum is required to be antitoxic or bactericidal—in other words, for use in Intoxications or Septicæmias and Local Processes tending to pass on into Septicæmias. The methods of preparing serums for use in these diseases have therefore to be conducted on totally different lines, serums required *against* intoxications being prepared by the inoculation into

animals of the toxins (separated by filtration, etc.) or dead cultures of the bacilli, while those wanted for Septicæmic Processes are best obtained by injecting animals with living cultures of micro-organisms rather than with dead toxic substances. In the former instance the serums are used to counteract the poisons of the micro-organisms by their antitoxic properties; in the latter their aim is—(*i*) to render the body unsuitable for the development of the micro-organisms, and (*ii*) to directly destroy the invading micro-organisms by the bactericidal power they possess. Antitoxic serum has been found of great value in Diphtheria, but unfortunately Tetanus is a disease which only shews itself after its poison has obtained a complete hold of the system, so that Tetanus Antitoxin is practically of no use for man in the treatment of acute cases, though undoubtedly of value in chronic cases. But in the other serums, *e.g.*, Anti-streptococcic, Anti-pneumococcic, and Anti-pest serums, in which bactericidal and not antitoxic power is required, very little such bactericidal power is found—as determined by Pfeiffer's sedimentation tests; while the enormous doses of the serum required—especially of Yersin's Anti-pest serum—and the fact that cases not only must be selected for their mildness, but also have to be treated by serum absolutely at the very beginning of the attack (in the majority of cases) cause these serums to be regarded by many as suspicious; while patients themselves often prefer to chance recovery from a mild attack than to undergo the extreme inconvenience of violent urticaria or continuous synovitis which their administration not unfrequently produces. There is, however, a great deal in the scientific value of such treatment, and it is to be hoped that reliable serums, capable of being accurately measured as to their immunising power and dosage, and constant in their effects, will shortly be forthcoming. The reason why such differences occur nowadays, not only in the dosage required, but also in the results obtained, lies probably in the fact that the cultures used at first to immunise the animal (which is presumed always to be quite healthy) from which the serum is obtained, are not uniform in their virulence through not having been grown at accurate temperatures or on perfectly prepared media for an equally definite time. In spite of the greatest care, most variable results are obtained under what, clinically, appear to be exactly the same conditions; while further, such serums as at present obtained are usually far too weak—requiring to be pushed to such an extent of dosage that it is often doubtful whether the remedy is not more harmful than the disease—and a further degree of concentration is imperative. All this, however, does not destroy the belief that curative serums are possible or the hope that reliable ones, possessing bactericidal power in a marked degree, constant in their action, and capable of being administered in reasonable doses, will be forthcoming for the treatment of Septicæmic Processes.

CHAPTER XII.

ON THE PREPARATION OF THE MORE COMMON CULTURE-MEDIA.

Nutrient Gelatine. Take 500 grms. of lean beef, free from fat, and chop it up into fine pieces. Place these in 1,000 c.c. of water and allow to stand for twenty-four hours. Squeeze the juice so obtained through a cloth. Add to the filtrate 10 grms. of Peptone (some use more, *e.g.*, 15 to 20 grms.), 5 grms. of Sodium Chloride, and 100 grms. Gelatine (more if necessary). Mix and heat carefully in a water bath, adding after a time concentrated solution of Sodium Carbonate, drop by drop, until alkalinity of the mixture is obtained as shewn by litmus paper. Sterilise, by heating two hours or so in the steam steriliser, or twenty minutes in the autoclave under 15 atmospheres' pressure. Next, clear, if necessary, by heating for half-an-hour, adding white of egg beaten up with its shell. Filter. While still liquid fill test tubes one-quarter to one-third full—taking care not to soil the sides—and sterilise these tubes. If required for needle cultures by stab, allow the tubes to stand upright; if for slanting surface cultures place the tubes on their side, the mouth being raised about 1 inch above the bottom which rests on the table.

Agar-Agar. Proceed as for Gelatine, only add 20 grms. of Agar-Agar powder instead of the 100 grms. Gelatine. If no Agar powder be procurable, soak 20 grms. of Agar-Agar in 1,000 c.c. of water overnight and proceed as with gelatine, filtering through flannel.

Glycerine-Agar. Five per cent. Glycerine is added to the nutrient Agar after the boiling and before filtration.

Solid Blood Serum. Blood is drawn off into a sterilised bottle (the first blood which comes from the animal being rejected) and allowed to stand for 24 to 30 hours, at the end of which time the clear serum is pipetted off into sterilised test-tubes, which are kept at 37°C. for a week—those shewing growths being discarded. Gelatine or Agar may be added at the first.

Loeffler's Blood Serum consists of $\frac{2}{3}$ fresh serum mixed with $\frac{1}{3}$ bouillon, to which 1 per cent. Grape Sugar has been added.

Bouillon is prepared as described in the preparation of broth for nutrient gelatine, only that the gelatine is omitted.

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